PHARMACOGNOSTICAL, PHYTOCHEMICAL AND PHARMACOLOGICAL STUDIES ON WHOLE PLANT OF *SIDA SPINOSA* LINN.

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CERTIFICATE

This is to certify that this dissertation entitled **"PHARMACOGNOSTICAL, PHYTOCHEMICAL AND PHARMACOLOGICAL STUDIES ON WHOLE PLANT OF *SIDA SPINOSA* LINN."** done by Mr. S. Selvadurai for the award of the degree of "**MASTER OF PHARMACY"** under **THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY, CHENNAI** is a bonafide work done by him in the Department of Pharmacognosy, Periyar College of Pharmaceutical Sciences for Girls, Tiruchirappalli. The work was performed under the guidance and supervision of **Dr. R. Senthamarai, M.Pharm, Ph.D**., Principal & Professor, Periyar College of Pharmaceutical Sciences for Girls, Tiruchirappalli.

This dissertation is submitted for acceptance as project for partial fulfillment of the degree of **"MASTER OF PHARMACY"** in Pharmacognosy, of the Tamilnadu Dr. M.G.R. Medical University, during September 2011.

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(**S.Selvadurai)**

CONTENTS

ABBREVIATIONS

AAS	:	Atomic Absorption Spectroscopy
ALP	:	Serum Alkaline Phosphatase
ANOVA	:	Analysis of Variance
BGL	:	Blood Glucose Level
CPCSEA	:	Committee for The Purpose of Control and Supervision of Experiments on Animals
FDA	:	Food and Drug Administration
TLC	:	Thin Layer Chromatography
GC-MS	:	Gas Chromatography –Mass Spectroscopy
GGT	:	Gama Glutamyl Transpeptidase
HPTLC	:	High Performance Thin Layer Chromatography
ICDRA	:	International Conference on Drug Regulatory Authorities
ICMR	:	Indian Council of Medical Research
IR	:	Infra Red Spectroscopy
IUPAC	**:**	International Units for Pure and Applied Chemistry:
NAPQ	:	N-Acetyl-*P*- Benzoquinoneimine.
OECD	:	Organisation for Economic Co- operation and Development
p.o	**:**	per oral
R_f	**:**	Retention factor
RT	**:**	Retention Time

SEM	:	Standard Mean Error
SGOT	:	Serum Glutamate Oxaloacetate Transaminase
SGPT	:	Serum Glutamate Pyruvate Transaminase
SN	**:**	Separation Number
TBA	:	Tertiary Butyl Alcohol
TCM	:	Traditional Chinese Medicine
T.S.	:	Transverse Section
UV	**:**	Ultra Violet
WHO	:	World Health Organisation

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INTRODUCTION

Herbal Medicine

Herbal medicine also called botanical medicine or phytomedicine refers to using a plant's seeds, berries, roots, leaves, bark, or flowers for medicinal purposes. Herbalism has a long tradition of use outside of conventional medicine. It is becoming more mainstream, as improvements in analysis and quality control along with advances in clinical research show the value of herbal medicine in treating and preventing disease.

Pharmacognosy

The word, "Pharmacognosy" is derived from two Greek words, "Pharmakon" means a drug and 'gnosis' means knowledge. Pharmacognosy is the subject that deals with the crude drugs obtained from vegetable, animal and mineral origin, modern aspects of the science include not only crude drugs but also their natural derivatives. Furthermore, the subject of pharmacognosy can also be expressed as an applied science, which deals with the biologic, biochemical and economical features of natural drugs and their constituents. Pharmacognosy deals with the knowledge of the history, source, distribution, cultivation, collection and preparation for the market, study of morphological characters, chemical constituents, therapeutic uses, substitution and adulteration of crude drugs. Crude drugs are the vegetable or animal drugs that consists of natural substances that have undergone only the process of collection and drying.

History of Pharmacognosy

It is very difficult to determine, since when crude drugs of plant and animal origin, as well as spices and condiments have been in use. No one will ever know what led primitive man, emerging from his ancestral origin, to select certain plant material for the treatment of various ailments though it may be assumed that during the long tradition from instinctive behaviour to more rational action there was a conscious realization that certain roots, leaves, barks, fruits and even plant exudation had some beneficial action. By trial and error, the primitive man must have acquired biologic knowledge that was useful in determining which plant and animal possessed food value and which were to be avoided as they were unpalatable or poisonous. Today there has accumulated a vast store of knowledge concerning the chemical nature of active constituents and therapeutic activities of different plant and crude drugs. **(Jeganathan, N.S., 2009)**

Why Herbal Medicine?

Herbal medicines are being used by about 80% of the world population primarily in the developing countries for primary health care. They have stood the test of time for their safety, efficacy, cultural acceptability and lesser side effects. The chemical constituents present in them are a part of the physiological functions of living flora and hence they are believed to have better compatibility with the human body. Ancient literature also mentions herbal medicines for age-related diseases namely memory loss, osteoporosis, diabetic wounds, immune and liver disorders, etc. for which no modern medicine or only palliative therapy is available.

These drugs are made from renewable resources of raw materials by ecofriendly processes and will bring economic prosperity to the masses growing these raw materials. **(Kamboj, V.P., 2000)**

Importance and Scope of Medicinal Plants

Green plants synthesis and preserve a variety of biochemical products, many of which are extractable and used as chemical feed stocks or as raw material for various scientific investigations. Many secondary metabolites of plants are commercially important and find use in a number of pharmaceutical compounds. However, a sustained supply of the source material often becomes difficult due to the factors like environmental changes, cultural practices, diverse geographical distribution, labour cost, and selection of the superior plant stock and over exploitation by pharmaceutical industries.

Herbs are staging a comeback and herbal 'renaissance' is happening all over the globe. The herbal products today symbolise safety in contrast to the synthetics that are regarded as unsafe to human and environment. Although herbs had been priced for their medicinal, flavouring and aromatic qualities for centuries, the synthetic products of the modern age surpassed their importance, for a while. However, the blind dependence on synthetics is over and people are returning to the naturals with hope of safety and security.

Over three-quarters of the world population relies mainly on plants and plant extracts for health care. More than 30% of the entire plant species, at one time or other, were used for medicinal purposes.

It is estimated that world market for plant derived drugs may account for about Rs.2,00,000 crores. Presently, Indian contribution is less than Rs.2000 crores. The annual production of medicinal and aromatic plant's raw material is worth about Rs.200 crores. This is likely to touch US $ 1150 by the year 2000 and US $ 5 trillion by 2050.

It has been estimated that in developed countries such as United States, plant drugs constitute as much as 25% of the total drugs, while in fast developing countries such as China and India, the contribution is as much as 80%. Thus, the economic importance of medicinal plants is much more to countries such as India than to rest of the world. These countries provide two third of the plants used in modern system of medicine and the health care system of rural population depend on indigenous systems of medicine of 2,50,000 higher plant species on earth, more than 80,000 are medicinal. India is one of the world's 12 biodiversity centres with the presence of over 45,000 different plant species. India's diversity is unmatched due to the presence of 16 different agro-climatic zones, 10 vegetation zones, 25 biotic provinces and 426 biotic (habitats of specific species). Of these, about 15,000 - 20,000 plants have good medicinal value.

However, only 7,000 – 7,500 species are used for their medicinal values by traditional communities. In India, drugs of herbal origin have been used in traditional systems of medicines such as Unani and Ayurveda since ancient times. The Ayurveda system of medicine uses about 700 species, Unani 700, Siddha 600, Amchi 600 and modern medicine around 30 species.

The drugs are derived either from the whole plant or from different organs like leaves, stem, bark, roots, flowers, seeds, etc. Some drugs are prepared from excretory plant products such as gums resins and latex. Even the Allopathic system of medicine has adopted a number of plant-derived drugs which form an important segment of the modern pharmacopoeia. Some important chemical intermediates needed for manufacturing the modern drugs are also obtained from plants (Eg. diosgenin, solasodine, b-ionone). Not only that plant-derived drug offers a stable market world wide, but also plants continue to be an important source for new drugs **(Thomas, J. 1997).**

Traditional systems of medicine continue to be widely practiced on many accounts. Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several allopathic drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments. Global estimates indicate that 80% of about 4 billion population afford the products of the Western Pharmaceutical Industry and have to rely upon the use of traditional medicines which are mainly derived from plant material.

This fact is well documented in the inventory of medicinal plants, listing over 20,000 species. Inspite of the overwhelming influences and our dependence on modern medicine and tremendous advances in synthetic drugs, a large segment of the world population still rely on drugs from plants.

In many of the developing countries the use of plant drugs is increasing because modern life saving drugs are beyond the reach of three quarters of the third world's population although many such countries spend 40 - 50% of their total wealth on drugs and health care. As a part of the strategy to reduce the financial burden on developing countries, it is obvious that an increased use of plant drugs will be followed in the future.

Among ancient civilizations, India has been known to be rich repository of medicinal plants. The forest in India is the principal repository of large number of medicinal and aromatic plants, which are largely collected as raw materials for manufacture of drugs and perfumery products. About 8,000 herbal remedies have been codified in Ayurveda. The *Rig-Veda* (5000 BC) has recorded 67 medicinal plants, *Yajurveda* 81 species, *Atharvaveda* (4500-2500 BC) 290 species, *Charka Samhita* (700 BC) and *Sushrut Samhita* (200 BC)

had described properties and uses of 1100 and 1270 species respectively, in compounding of drugs and these are still used in the classical formulations, in the Ayurvedic system of medicine **(Thomas, J., 1997).**

Unfortunately, much of the ancient knowledge and many valuable plants are being lost at an alarming rate. With the rapid depletion of forests, impairing the availability of raw drugs, Ayurveda, like other systems of herbal medicines has reached a very critical phase. About 50% of the tropical forests, the treasure house of plant and animal diversity have already been destroyed. In India, forest cover is disappearing at an annual rate 1.5/year. What is left at present is only 8% as against a mandatory 33% of the geographical area. Many valuable medicinal plants are under the verge of extinction **(Thomas, J., 1997).**

Ayurveda, Siddha, Unani and Folk (Tribal) medicines are the major systems of indigenous medicines. Among these systems, Ayurveda is most developed and widely practised in India. Ayurveda dating back to 1500 - 800 BC has been an integral part of Indian culture. The term comes from the Sanskrit root *Ayuh* (life) and *Veda* (knowledge). As the name implies it is not only the science of treatment of the ill but covers the whole gamut of happy human life involving the physical, metaphysical and the spiritual aspects **(Thomas, J., 1997).**

Ayurveda recognises that besides a balance of body elements one has to have an enlightened state of consciousness, sense organs and mind if one has to be perfectly healthy. Ayurveda by and large is an experience with nature and unlike in Western medicine, many of the concepts elude scientific explanation. Ayurveda is gaining prominence as the natural system of health care all over the world.

Today this system of medicine is being practised in countries like Nepal, Bhutan, Sri Lanka, Bangladesh and Pakistan, while the traditional system of medicine in the other countries like Tibet, Mongolia and Thailand appear to be derived from Ayurveda.

Phytomedicines are also being used increasingly in Western Europe. Recently the US Government has established the "Office of Alternative Medicine" at the National Institute of Health at Bethesda and its support to alternative medicine includes basic and applied research in traditionalsystems of medicines such as Chinese, Ayurvedic, etc.

with a view to assess the possible integration of effective treatments with modern medicines. The development of systematic Pharmacopoeias dates back to 3000 BC, when the Chinese were already using over 350 herbal remedies. Ayurveda, a system of herbal medicine in India, Sri Lanka and South-East Asia has more than 8000 plant remedies and using around 35,000 - 70,000 plant species. China has demonstrated the best use of traditional medicine in providing the health care.

China has pharmacologically validated and improved many traditional herbal medicines and eventually integrated them in formal health care system. Plants, especially used in Ayurveda can provide biologically active molecules and lead structures for the development of modified derivatives with enhanced activity and /or reduced toxicity. The small fraction of flowering plants that have so far been investigated have yielded about 120 therapeutic agents of known structure from about 90 species of plants.

Some of the useful plant drugs include vinblastine, vincristine, taxol, podophyllotoxin, camptothecin, digitoxigenin, gitoxigenin, digoxigenin, tubocurarine, morphine, codeine, aspirin, atropine, pilocarpine, allicin, curcumin and ephedrine among others **(Thomas, J., 1997).**

In some cases, the crude extract of medicinal plants may be used as medicaments. On the other hand, the isolation and identification of the active principles and elucidation of the mechanism of action of a drug is of paramount importance.

Hence, works in both mixture of traditional medicine and single active compounds are very important. Where the active molecule cannot be synthesised economically, the product must be obtained from the cultivation of plant material. About 121 (45 tropical and 76 subtropical) major plant drugs have been identified for which no synthetic one is currently available. The scientific study of traditional medicines, derivation of drugs through bioprospecting and systematic conservation of the concerned medicinal plants are thus of great importance **(Sharma, *et al.*, 1991).**

A major lacuna in Ayurveda is the lack of drug standardisation, information and quality control. Most of the Ayurvedic medicines are in the form of crude extracts which are a mixture of several ingredients and the active principles when isolated individually fail to give desired activity. This implies that the activity of the extract is the synergistic effect of its various components.

In the absence of Pharmacopoeia data on the various plant extracts, it is not possible to isolate or standardise the active contents having the desired effects. Ayurvedic Pharmacopoeia compiled on modern lines and updated periodically is an urgent requirement **(Sharma, *et al.*, 1991).**

A combination therapy integrating Ayurveda and Allopathy whereby the side effects and undesirable reactions could be controlled can be thought studies can show that the toxic effects of radiations and chemotherapy in cancer treatment could be reduced by Ayurvedic medications and similarly surgical wound healing could be accelerated by Ayurvedic medicines.

Modern science and technology have an essential role to play in the process **(Sharma, *et al.*, 1991).** Despite the diverse nature of crops grown in the country and the existence of a fast growing pharmaceutical sector, the share of India in world trade is quite insignificant considering the large geographical area. However, this is bound to rise rapidly with better research inputs and efficient management of the farm sector. So far, India has been involved in the export of only large volume of raw material. To achieve competitive advantage we need to resort to low volume high cost (value) trade through value addition to the raw and unfinished products.

It is therefore, necessary to develop genetically superior planting material for assured uniformity and desired quality and resort to organised cultivation to ensure the supply of raw material at grower's end. Post harvest storage and process technologies need to be developed to produce the value added finished products that may be directly utilised by the industry **(Sharma, *et al.*, 1991).**

Role of WHO in Herbal Medicine

WHO defines Herbal Medicines as "finished, labelled, medicinal products that contain as active ingredients, aerial or underground parts of plants or other plant material or combinations thereof, whether in the crude state or as a plant preparation".

Another resolution taken by the WHO supports herbal medicine as being of "great importance" to the health of the individual and their communities. It is estimated that 80 percent of the world's population relies on traditional medicines for a significant part of their health care.

Annual sales of herbal products were 1 billion in 1991, and in 1997, 3.24 billion in the USA. The interest of herbal medicine has increased immensely in the last few years, which is great; unfortunately the herbal knowledge has not increased as rapid as the sales, meaning that there is a great number of people using herbs without the appropriate level of knowledge to use them, not only safely but effectively.

Most of us know people using herbal medicines just because they were recommended by a friend or relative, creating an unsafe and ineffective aura for herbal remedies, which is capitalized by groups against herbal remedies in their blind pursuit to ban herbs or to control their sales and use. This creates an atmosphere of mistrust, and does not benefit any type of medicine, allopathic or traditional.

Therefore our responsibility is to learn the use of herbs to treat our conditions as well as our family's safety, and the effectiveness of herbs depends on the knowledge of when and how to use them.

Standardization of Herbal Medicine

In indigenous/traditional systems of medicine, the drugs are primarily dispensed as water decoction or ethanolic extract. Fresh plant parts, juice or crude powder are a rarity rather than a rule. Thus medicinal plant parts should be authentic and free from harmful materials like pesticides, heavy metals, microbial or radioactive contamination, etc.

The medicinal plant is subjected to a single solvent extraction once or repeatedly, or water decoction or as described in ancient texts. The extract should then be checked for indicated biological activity in an experimental animal model(s). The bioactive extract should be standardized on the basis of active principle or major compound(s) along with fingerprints. The next important step is stabilization of the bioactive extract with a minimum shelf-life of over a year. The stabilized bioactive extract should undergo regulatory or limited safety studies in animals. Determination of the probable mode of action will explain the therapeutic profile.

The safe and stable herbal extract may be marketed if its therapeutic use is well documented in indigenous systems of medicine, as also viewed by WHO. A limited clinical trial to establish its therapeutic potential would promote clinical use. The herbal medicines developed in this mode should be dispensed as prescription drugs or even OTC products depending upon disease consideration and under no circumstances as health foods or nutraceuticals **(Kamboj, V.P., 2000).**

Diabetes Mellitus

Diabetes is one of the most challenging diseases facing health care professionals today. Its increasing prevalence puts a large burden on society and the public health sector **(Leroith, D., *et al.*, 2005)**. Type 1 diabetes is characterized by an absolute deficiency of insulin secretion, associated with auto-immune destruction of pancreatic β-cells, and this disease is more likely to occur in relatives of an affected person **(Bottini, N., *et al.*, 2006).** Type 2 diabetes, which accounts for more than 90% of cases, is caused by a combination of resistance to insulin action and impaired insulin secretion **(Warren, RE., *et al.*, 2004).**

Plants have been reported as an exemplary source of drugs, and many of the currently available drugs have been derived directly or indirectly from them. In recent years, there has been renewed interest in plant medicine **(Dubey, GP., *et al.*, 1994; Prince, PS., *et al.*, 1998; Ladeji, O., *et al.*, 2003)** for the treatment against different diseases as herbal drugs are generally out of toxic effect **(Geetha, BS., *et al.*, 1994; Rao, BK., *et al.*, 2003)** reported from research work conducted on experimental model animal.

Although in human, whether there is any toxic effect are not investigated. It is reported that about 800 plants may possess anti-diabetic potential **(Grover, JK., *et al.*, 2002).** Isolated studies screened various plants having "folk medicine reputation" by biochemical test for this antidiabetogenic effect **(Vats, V., *et al.*, 2002)**

Plants having hypoglycemic activity (Nahar, N., 1993)

Diabetes mellitus is wide spread disorder, which has long been in the history of medicine. Before the advent of insulin and oral hypoglycaemic drugs the major form of treatment involved the use of the plants. The following are some of the plants reported in the treatment of diabetes.

Plant	**Plant Parts**	**Type of Test Sample**
Trigonella foenum-gracecum	Seed	Alcohol, Aqueous Extract
Nephoelepsis tuberose	Bulb	Juice
Costus specious	Rhizome	Juice
Plantago ovata	Husk	Powder
Allium sativum	Bulb	Juice
Hemidesmus indicus	Root	Alcoholic Extract
Allium ceba	Bulb	Juice

The Natural Way

A healthy diet, preferably containing mostly organic ingredients, is a good place to start – as is the maintenance of a regular fitness program geared to your body needs. Pacing yourself and making time to unwind and practice stress management and relaxation techniques can also go a long way to ensuring harmony and balance in the brain and nervous system.

***Sida spinosa* Linn.** is a traditional medicinal plant belonging to family Malvaceae. It is found in a variety of distributed habitats including empty lots, fields and road side.

The plant ***Sida spinosa*** Linn**.** has been claimed to possess various medicinal properties. The root, leaves and fruits destroy "Kapha" and "Vata" tonic in wasting diseases, cure ulcers and biliousness, useful in urinary discharges, scalding urine, leprosy, and skin infections, the fruit is also astringent and cooling. The whole plant of *Sida spinosa* Linn. has been claimed to possess the hypoglycemic activity.

In this context the present work has been attempted to investigate the **"Pharmacognostical, Phytochemical and Pharmacological Studies on Whole Plant of *Sida spinosa* Linn.**

LITERATURE REVIEW

Earlier Literature Review of *Sida spinosa* Linn. and other *Sida* Species are listed below

- Faten M. M Darwish and Manfred G. Reinecke (2003) identified the Ecdysteroids and other constituents from *Sida spinosa* L.
- Jabeen, F., *et al.*, (1996) studied the structure and distribution of mucilage cells in leaf epidermis of Malvales.
- Rao, K. S., *et al.*, (1997) evaluated the Anti-inflammatory and hepatoprotective activities of *Sida rombifolia* Linn.
- Alam, M., *et al.*, (1998) reported on Standardisation studies on Ksirabala tailam.
- Rao, K. S., *et al.*, (1998) evaluated on Antihepatotoxic activity of *Sida cordifolia* whole plant.
- Franzotti, E. M, *et al., (*2000) screened the anti-inflammatory, analgesic activity and acute toxicity of *Sida cordifolia* L.
- Mani Raj, N., *et al.*, (2000) reported the Biochemical characterization of medicinal plants in the wild and domestic environment.
- Tahiliani, P., *et al.*, (2000) screened the Relative roles of some plant extracts in the regulation of serum thyroid hormones and glucose concentration in female rats.

- Kotoky, J., *et al.*, (2001) evaluated the Hepatoprotective activities of *Sida cordifolia* L. root against carbon tetrachloride intoxicated rats.

- Raj, N. M., *et al.*, (2001) studied the Biochemical characterization of medicinal plants in the wild and domestic environment.

- Suneetja, M. S., *et al.*, (2002) reported the Trade in medicinal plants in kerala-issues, problems and prospects.

- Varier, P. M., *et al.*, (2002) reported the Effect of Kerala system of Svedana Kriyas in degenerative diseases.

- Ekramul Islam, M., *et al.*, (2003) studied the Cytotoxicity and antibacterial activity of *sida rombifolia* (Malvaceae) grown in Bangladesh.

- Seal, S., *et al.*, (2003) screened the effect of Na-dikegulac on growth, biomass and alkaloid yield in *Sida acuta.*

- Chatterjee, S., *et al.*, (2005) reported the Evaluation of the efficacy and safety of "Nourishing baby oil" in infantile xerosis.

- Maragatham, C., and Panneerselvam, A., (2010) In vitro studies on the effect of precursors for the production of secondary metabolites in *Sida cordifolia*.

- Mishra, A. N., *et al.,* (2010) studied the chemical constituents and antioxidant activity of *Sida cordifolia* Linn.

NEED FOR PRESENT STUDY

The plant ***Sida spinosa*** Linn**.** has been claimed to possess various medicinal properties. The root, leaves and fruits destroy "Kapha" and "Vata" tonic in wasting diseases, cure ulcers and biliousness, useful in urinary discharges, scalding urine, leprosy, and skin infections. The fruit is also astringent and cooling **(Gunatilaka, *et al.*, 1980; Lutterodt., 1988).**

Sida spinosa Linn. is used in the treatment of asthma and other chest ailments and as a tonic **(Prakash, *et al.*, 1981).** The leaves have reportedly been used for treatment of some skin diseases and as oral snake bite treatment **(Iwu., 1993).**

The roots and leaves of *Sida spinosa* Linn. are used in treatment of diarrhea and dysentery **(Noumi and yomi., 2001).**

The leaves are demulcent and refrigerant, and are useful in cases of gonorrhoea, gleet and scalding urine **(Kirtikar and Basu., 1999).**

The decoction of the root-bark and root is used as a demulcent in irritability of the bladder and in gonorrhoea **(Rudel, *et al.*, 1992; coll, *et al.*, 1994).**

The root acts as a gentle tonic and diaphoretic, and is complied in mild cases of debility and fever **(Wealth of India, 1999).**

The present investigation is concerned with the widely distributed indigenous medicinal plant ***Sida spinosa*** Linn. From the survey and relevant literature of the plant ***Sida spinosa*** Linn**.** it is most well known in traditional medicinal practices and less work has been performed on different parts of the plant.

The present study has been undertaken to evaluate the **Pharmacognostial, Phytochemical, Pharmacological aspects of the whole plant of *Sida spinosa* Linn.**

PLAN OF WORK

- Collection and Authentication of *Sida spinosa* Linn.
- Shade drying and Pulverization

PHARMACOGNOSTICAL STUDIES OF *SIDA SPINOSA* LINN.

- Macroscopy
- Microscopy

Transverse Section of

- Leaf
- Petiole
- Stem
- Root
- **Quantitative Microscopy**
- Vein islet number
- Vein termination number
- Stomatal number
- Stomatal index
- Length and Width of Phloem fibres
- Powder Microscopy

Fluorescence Analysis of *Sida spinosa* Linn.

- Analysis of Extracts
- Analysis of Powder drug

Physico Chemical Standards

- **Ash Values**
  - Total ash
  - Acid insoluble ash
  - Water soluble ash
  - Sulphated ash
- **Extractive Values**
  - Alcohol soluble extractive
  - Water soluble extractive
- **Loss on Drying**
- **Determination of Crude Fibre Content**

Phytochemical Studies of *Sida Spinosa* Linn.

Extraction of Plant material

Successive Solvent extraction of air dried Whole Plant of *Sida spinosa* Linn. of Non – polar to polar solvents.

Preliminary Phytochemical Evaluation of Extracts

- **Separation and Identification of Isolated Plant Constituents**

- **Chromatographic Methods**
  - Thin Layer Chromatography (TLC)
  - Column Chromatography (CC)
  - High Performance Thin Layer Chromatography (HPTLC)
- **Characterisation of Isolated Plant Constituents**
  - Infra Red Spectroscopy (IR)
  - GC - MS

PHARMACOLOGICAL STUDIES

- Acute Toxicity Studies
- Hypoglycemic Activity
- Antimicrobial Activity

RESULTS & DISCUSSION

CONCLUSION

PHARMACOGNOSTICAL INVESTIGATIONS

Scientific Classification

Division	-	Angiospermae
Class	-	Dicotyledon
Sub class	-	Archichlamydeae
Order	-	Malvales
Family	-	Malvaceae
Genus	-	*Sida*
Species	-	*spinosa*

Macroscopy

Synonym	-	*Indian mallow, Sida alba* (Charles Bixler, 2003)
Biological Source	-	*Sida spinosa*
Family	-	Malvaceae

Vernacular Names (Khare, C.P., 2007)

English	-	Prickly sida
Hindi	-	Gulsakari, Barriyara
Kannadam	-	Kaadu menthya
Malayalam	-	Kattuventiyam
Tamil	-	Mairmanikkam, Arivalmanai poondu
Telugu	-	Nagabala, Teranella

Description (The Wealth of India, 1999; Warrier, P.K., *et al.*, 1996)

Erect, annual or perennial, stellate, 30 cm to 1 m tall under shrub. Leaves with filiform, 2 – 5 mm long stipules, petiole 2 – 20 mm long, 1 – 3 spiny tubercles present on stem at the base of petiole, lanceolate to ovate, oblong or somewhat orbicular, round at base, acute or obtuse at apex, serrate. Flowers axillary, solitary or 2 – 5 in fascicles in terminal branches, pedicel 2 – 5 mm, in fruit upto 0.2 cm long, joined near the middle or top. Calyx 4 – 5 mm long. Fruit depressed globose, pubescent above, mericarps 5, membranous, 2 – 3 mm long. Seeds 1.5 mm long, glabrous, brown to black.

Distribution

Michigan in the West to Massachusetts in the East South to Florida and Texas. Also in tropical America. In India commonly found in Tamil Nadu, Kerala, Andraapradesh, Karnataka.

Habit & Habitat (Nadkarni, A.K., 1976)

Throughout the hotter parts of India, ascending to an altitude of 1350 m. It is found in a variety of distributed habitats including empty lots, fields and road side **(Boulos and Hadidi., 1984).**

Leaves

Leaves with filiform, 2 – 5 mm long stipules, petiole 2 - 20 mm long, 1 - 3 spiny tubercles present on stem at the base of petiole. **(Kirtikar & Basu., 1999)**

Flowers

Flowers pale yellow or white, auxiliary **(The Wealth of India, 1999).**

Seeds

Seeds 1.5 mm long, glabrous, brown to black **(The Wealth of India, 1999).**

Powder Characters

Appearance	:	Moderately coarse powder
Colour	:	Pale green
Odour	:	Characteristic
Taste	:	Slightly bitter

MICROSCOPY

Collection of Specimens

The plant specimen for the proposed study were collected from Komaneri village, Thothukudi Dist. Tamilnadu in the month of January 2011, the plant material was identified and authenticated by Dr. P. Jayaraman, Plant Anatomy Research Centre, Pharmacognosy Institute, Chennai. Care was taken to select healthy plants and normal organs. The required samples of different organs were cut and removed from the plant and fixed in FAA (Formalin - 5ml + Acetic acid - 5ml + 70% Ethyl alcohol - 90ml). After 24 hours of fixing, the specimens were dehydrated with graded series of tertiary - butyl alcohol. Infiltration of the specimen was carried by gradual addition of paraffin wax (melting point 58^{o} - 60^{o}C) until TBA solution attained super-saturation. The specimen was cast into paraffin blocks **(Johansen, 1940; O'Brien, *et al*., 1964).**

Sectioning

The paraffin embedded specimens were sectioned with the help of Rotary Microtome. The thickness of the section was 10-12 µm. Dewaxing of the section was by customary procedure **(Johansen, 1940).** The sections were stained with Toludine blue **(O'Brien, *et al.*, 1964).** Since Toludine blue is a polychromatic stain, the staining results were remarkedly good; and some cytochromatic reactions were also obtained. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc. Wherever necessary sections were also stained with Safranin and Fast green and IKI for starch.

For studying the stomatal morphology, venation pattern and trichome distribution, paradermal sections (sections taken parallel to the surface of leaf) as well as clearing of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey's maceration fluid were prepared **(Sass, 1940).** Glycerin mounted temporary preparations were made for macerated/cleared materials.

Powdered materials of different parts were cleared with sodium hydroxide and mounted in glycerine medium after staining. Different cell components were studied and measured (**Metcalfe, C.R., 1950).**

Photomicrographs

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photography of different magnifications were taken with Nikon Lab photomicrographic units.

For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have bifringent property, under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale-bars. Descriptive terms of the anatomical features are as given in the standard Anatomy books **(Easu, 1964).**

Leaf T.S

A) Midrib T.S (Fig - 6. 6.1)

The midrib is 350 µm in vertical plane and 350 µm in transverse plane, the midrib consists of bowl shaped abaxial part and slightly convex on the adaxial side of the midrib, the adaxial epidermal cells are small, cubical and thick walled; the palisade parenchyma of the lamina tranverse upto the adaxial part of the midrib, and the ground tissue is inconspicuous in the adaxial state, the ground tissue on the aboxial region of the midrib consist of outer two or three layers of thick walled, compact, angular collenchyma cells and inner three or four layers of thin walled parenchymatous ground tissue **(Fig 7.2).**

The vascular strand of the midrib occurs as an abaxial arc, the vascular bundle is collateral, consisting of xylem elements in radical files on the adaxial side and phloem elements as narrow strip on the abaxial part of the midrib, the xylem elements are circular, thick walled and occur in multiples of three or four elements, the phloem on the abaxial side consist of sclerenchyma sheath, the vascular bundle of the midrib is supported vertically by thick walled parenchyma cells abutting the adaxial epidermis **(Fig - 6, 6.1).**

B) Lamina (Fig - 7, 7.1, 7.2)

The lamina is 150 µm thick. The leaf is dorsiventral, mesomorphic, hypostomatic, the adaxial epidermis is single layered and consist of squarish to rectangular; thick walled, epidermal cells, the abaxial epidermal cells are small, cubical and thick walled, palisade mesophyll cells are cylindrical, compact and occupy two third thickness of the lamina, palisade cells are 50 µm in height, spongy mesophl tissue consist of thin walled, spherical cells, two or three layered; less compact **(Fig 7.2**) lateral vein bundles are prominent and has shallow bowl shaped abaxial part and a flat adaxial side of the lamina and phloem on the abaxial side**, (Fig 7.1)** stellate trichomes are evident on abaxial surface of the lamina **(Fig - 7.3).**

C) Stomatal Morphology (Fig - 8, 8.1)

The abaxial epidermis is stomatiferous, the epidermal cells are thick walled and have wavy anticlinical wall, the stomata is anomocytic (irregular epidermal cells which are indistinguishable from other epidermal cells. i.e; subsidiary cells are absent. The guard cells are 15 x 25 µm in size.

D) Petiole T.S (13, 13.1)

The petiole is cordate shaped in cross sectional outline, with wide cup shaped abaxial part and a flat adaxial side, the epidermis of the petiole is single layered and the epidermal cells are small, cubical, compact and thick walled; the ground tissue of the petiole is differentiated into outer three or four layers of thick walled, angular, compact, collenchyma cells and inner thin walled, elliptical parenchymatous ground tissue **(Fig. 9.1).**

The vascular strand of the midrib comprises a median vascular bundle and two lateral vascular bundles one on either side of the median bundle; the vascular bundles are collateral and are separated by the narrow strips of parenchymatous ground tissue; the median bundle consists of parallel lines of thick walled xylem elements on the adaxial side and a narrow band of phloem around the xylem on the abaxial side; the lateral bundles have radical files of xylem elements facing the centre of the petiole and phloem around the xylem on the outer side; the phloem of the vascular bundles have sclerenchymatous sheath is present around the phloem **(Fig - 9.2).**

E) Venation Pattern (Fig – 10, 11, 11.1, 12)

The venation pattern of the lamina shows the vein islets and vein termination; in *Sida spinosa* the vein islets are rectangular or square shaped and wide; the vein terminations are fairly distinct, short and branched onces or unbranched, the branched termination extend upto the middle portion of the vein islet. The extreme ends of the vein terminals bear small cluster of elongated tracheids **(Fig 9.1).**

F) Trichome Morphology (Fig 6.1)

Sida spinosa exhibits two types of trichomes, i.e. glandular and non-glandular trichomes are stellate and consists of a short stalk with free branches of various lengths has dagger like pointed tip, (Fig 7.2) the glandular trichomes are multi cellular, linear, un-branched, having a short stalk and thin walled cells arranged one above the other.

Crystals (Fig. 6.2,)

When the midrib and lamina are viewed under polarized light, the calcium oxalate crystals and lignified cells appear bright; in midrib the crystals are mostly distributed in the phloem region and in the lamina crystals are evident in the mesophyll tissue. The crystals are druses or sphaero crystals type. The crystals are 20 µm wide.

G) Stem T.S (Fig – 14, 14.1)

The stem is circular in cross section; the stem shows normal secondary growth; the stem has thick hollow, continuous cylinder of secondary xylem and secondary phloem. No periderm is found. Epidermis is thick and unistratose; the cells are cubical and intact and thick walled; cortex is narrow; the epidermis is followed by one or two layers of thick walled sclerenchyma cells and inner to this region four or five layers of thick walled collenchyma cells are present; the inner boundary of the cortex is delimited by perivascular sclerenchyma cells.

The vascular system of the young stem occurs in a continuous cylinder of secondary xylem surrounded by secondary phloem **(Fig 11.1).** The xylem elements are present in radical files of three or four elements in parallel with xylem fibres; the vessels are narrow, circular or elliptical and thick walled **(Fig 11.1)**. The phloem occur as a narrow strip around the xylem cylinder; pith is wide and consists of thin walled polygonal shaped parenchyma cells; starch grains are abundant in the pith cells of the stem **(Fig 12.2).**

Root T.S (Fig 11.1, 2; 12.1,)

In cross sectional outline the root is circular and shows secondary growth **(Fig 11.1)**. The epidermis is replaced by a narrow zone of periderm; periderm is superficial in origin; the periderm is 50 µm in thickness; periderm consists of a 4 to 5 layers of suberised, tabular phellem cells which occur in radical series **(Fig 11.2)** and inner 1 or 2 layers of phelloderm cells; fissures of various shape are evident in the periderm; periderm is followed by cortex, the cortical cells are thin walled and tangentially stretched and 5 to 6 layered; perivascular sclerenchymatous fibres are randomly distributed in the cortex; **(Fig 14.1)** the secondary xylem occur as dense solid cylinder; secondary xylem elements radical from the centre of the root, vessel elements are circular, wide thick walled and mostly solitary and accompanied by the xylem fibres **(Fig 14.2)** primary xylem elements are present in the centre of the root; secondary phloem occur as a narrow band around the xylem cylinder.

I) Maceration (Fig - 13, 14 and 15)

Macerated tissue shows the individual cells in three dimensional view; in *Sida spinosa* vessels are two type, one type is narrow lumened and long, the other type of vessel element is short, and wide lumened both types have pitted secondary wall thickenings; perforation plate is simple; vessels length range from 300 µm wide, vessel element to 500 µm narrow elements and width messures about 20 µm to 40 µm; fibres are long, narrow, thick walled and lignified with pointed tips; the fibres have thick lignified walls and narrow lumen.

Pits are not evident in the fibres. Some of the vessel elements have short tail and oblique perforations **(Fig 14.1,2)** The short vessel elements are tail less and have horizontal perforation **(Fig 15.1).**

Quantitative Microscopy

Quantitative Microscopy includes the vein islet number, vein termination, stomatal number and stomatal index were determined on fresh leaves using standard procedures **(Kokate, C.K. 1994).**

A. Vein islet and Vein termination Number

- Vein islet is used to denote the minute area of photosynthetic tissue encircled by the ultimate divisions of the conducting strands. The number of vein islet per sq.mm. area was calculated as vein islet number.
- Vein termination number may be defined as the number of vein terminals present in one sq.mm. area of the photosynthetic tissues.

Determination of Vein islet and Vein termination Number

- Pieces of leaves were cut from the various regions of the leaves between midrib and margin, cleared in chloral hydrate, stained and mounted on a slide.
- Camera lucida and drawing board were arranged with the help of a stage micrometer, and 1mm square was drawn on the paper. Then the stage micrometer was replaced by the preparation and the veins were traced in the square, the vein-islet and vein–terminations were counted in the square. Five such readings were taken and the average was calculated.

B. Stomatal Number & Stomatal Index

- Stomatal number is defined as the number of stomata present in one square mm of the photosynthetic tissues.

Determination of Stomatal Number

- Pieces of upper and lower epidermal peelings were mounted on a slide. With the help of camera lucida and stage micrometer, 1mm square was drawn on a paper.

- The stage micrometer was replaced by the preparation. Then the preparation was observed and the stomata were marked in that unit area. Number of stomata present in that unit area was calculated.

- Five readings were taken, the range of stomatal number was calculated separately for both upper and lower epidermis.

Stomatal Index

It is the percentage which the number of stomata forms to the total number of epidermis cells, each stoma being counted as one cell.

$$\text{Stomatal Index} = \frac{S}{S+E} \times 100$$

where,

S = Number of stomata per unit area

E = Number of epidermal cells in the same unit area

Determination of Stomatal Index

The procedure adopted in the determination of stomatal number was observed under high power (45X). The epidermis cells and the stomata were counted. From these the stomatal index was calculated using the above formula.

C. Palisade Ratio

The average number of palisade cells beneath each epidermal cell is termed as the palisade ratio.

Determination of Palisade Ratio

- Pieces of leaves were cut off from various regions between the midrib and the margin.
- These pieces of leaves were cleared and mounted on a slide. Camera lucida and drawing board were arranged.
- Then the slide was observed under high power. Four continuous epidermal cells devoid of trichomes and stomata were traced.
- The total number of palisade cells were counted and divided by four.

Five such readings were taken and the average was calculated. The results are shown in **Table 1**

Table - 1

Data showing the Quantitative Microscopy of Whole Plant of *Sida spinosa* Linn.

S.No	Vein Islet Number	Vein Termination Number	Stomatal Number (Upper Epidermis)	Stomatal Number (Lower Epidermis)	Stomatal Index (Upper Epidermis)	Stomatal Index (Lower Epidermis)	Palisade Ratio (Upper Epidermis)
1.	9 .0	12.0	19.0	21.0	15.6	11.0	3.9
2.	11.0	9.0	21.0	17.0	14.4	12.1	4.1
3.	8.0	7.0	18.0	19.0	17.2	11.4	4.0
4.	9.0	11.0	16.0	23.0	13.6	12.1	3.8
5.	10.0	9.0	19.0	21.0	14.8	12.3	3.6
Minimum	8.0	7.0	16.0	17.0	13.6	11.4	3.6
Average	9.4	9.6	18.6	20.2	15.12	11.78	3.88
Maximum	11.0	12.0	21.0	23.0	17.2	12.3	4.1

Physico - Chemical Standards (Indian Pharmacopoeia, 1996)

Ash values

Ash values such as total ash, acid insoluble ash, water-soluble ash, and sulphated ash were determined according to Indian Pharmacopoeia. The ash value is the criteria to judge the purity or identity of powdered drug. The ash content of the drug can be determined by incinerating the powder drug so as to burn all organic matter the residue, which remains after incineration is known as ash content/value. The ash consists of inorganic salt that occurs maturely (or) sometimes added deliberately during adulteration **(Patel, N.M., *et al.*, 2007; Gupta, M.K., *et al.*, 2007).** For determination of whole plant powder and sifted through sieve no. 20 and following tests were performed.

Determination of Total Ash

About 2 g each of powdered drug was accurately weighed and taken separately in silica crucible, which was previously ignited and weighed. The powder was spread as a fine layer on the bottom of crucible. The powder was incinerated gradually by increasing temperature to make it dull red hot until free from carbon. The crucible was cooled and weighed. The percentage was repeated to get constant weight.

Determination of Water Soluble Ash

The total ash was boiled with 25 ml water and filtered through an ashless filter paper (Whatmann - 41). It was followed by washing with hot water. The filter paper was ignited in the silica crucible, cooled and the water insoluble matter was weighed. The water-soluble ash was calculated by subtracting the water insoluble matter from the total ash.

Determination of Acid Insoluble Ash

The ash obtained as described above was boiled with 25 ml of 2N HCl for 5 minutes. The insoluble ash was collected on an ashless filter paper and washed with hot water. The insoluble ash was transferred into a crucible, ignited and weighed. The procedure was repeated to get a constant weight. The percentage of acid insoluble ash was calculated with reference to the air-dried drug.

Determination of Sulphated Ash

A silica crucible was heated to red heat for 10 minutes and was allowed to cool in a desiccator and weighed. A gram of substance was accurately weighed and transferred to the crucible. It was ignited gently at first, until the substance was thoroughly charred. Then the residue was cooled and moistened with 1 ml of concentrated sulphuric acid, heated gently until white fumes are no longer evolved and ignited at 800 ^{o}C ± 25 ^{o}C until all black particles have disappeared. The ignition was conducted in a place protected from air currents. The crucible was allowed to cool. A few drops of concentrated sulphuric acid were added and heated. Ignited as before and was allowed to cool and weighed.

Determination of Loss on Drying

About 2g of the powdered drug of *Sida spinosa* was accurately weighed in a tared dish and dried in air oven at 100^{o}C. It is cooled in a desiccator and again weighed. Loss on drying was calculated with reference to the amount of air dried powder.

Determination of Alcohol Soluble Extractive

About 5g of the powdered drug was macerated with 10 ml of alcohol of the specified strength in a closed flask for 24 hours, shaking frequently during 6 hours and allowed to stand for 18 hours. It was filtered rapidly taking precaution against loss of alcohol. Then 25 ml of the filtrate was evaporated to dryness in a tared flat bottomed shallow dish dried at 105^{o}C and weighed. The percentage of alcohol soluble extractive was calculated with reference to the air dried powder.

Determination of Water Soluble Extractive

About 5g of the powdered drug was added to 50 ml of water at 80^{o}C in a stoppered flask. Shaken well and allowed to stand for 10 minutes, cooled to 15^{o}C and to it, 2 gram of kieselghur was added and filtered. 5ml of the filtrate was transferred to a tared evaporating basin 7.5 cm in diameter. The solvent was evaporated on water bath, drying is continued for half an hour. Finally, it was dried in a steam oven for 2 hours and weighed. The percentage of water soluble extractive was calculated with reference to the air dried drug.

Determination of Crude Fibre Content (Dutch Process)

About 2g of drug was weighed accurately and transferred to a porcelain dish. 50 ml of 10% nitric acid was added, boiled for 30 seconds with constant stirring and filtered through fine mesh cotton cloth. The residue was washed with 5ml of boiled water. The material from the cloth was collected in a porcelain dish boiled with 50 ml of 2.5% caustic soda. Then the liquid was filtered with 100 ml of boiling water. The fibres were collected in a water bath, dried at 105^{o}C and weighed. From the weight of residue, crude fibre content was calculated.

The results of the above parameters are shown in **Table 2**

Table - 2

Data Showing the Physico – Chemical Standards of *Sida spinosa* Linn.

S.No	Total Ash %w/w	Water Soluble Ash %w/w	Acid Insoluble Ash %w/w	Sulphated Ash %w/w	Loss on Drying %w/w	Alcohol Soluble Extractive % w/w	Water Soluble Extractive % w/w	Crude Fibre Content % w/w
1.	5.10	3.47	1.05	3.92	5.76	6.00	9.40	12.50
2.	5.62	4.15	0.94	3.89	5.21	6.57	9.24	15.01
3.	4.91	3.92	0.91	4.53	5.92	5.93	8.97	13.90
4.	5.38	4.13	1.16	3.94	5.53	6.21	9.32	14.74
5.	5.81	3.79	1.68	3.69	5.18	7.03	9.01	14.31
Minimum	4.91	3.47	0.91	3.69	5.21	5.93	8.93	12.50
Average	5.36	3.89	1.15	3.89	5.72	6.35	9.19	14.09
Maximum	5.81	4.15	1.68	4.53	6.17	7.03	9.40	15.01

FLUORESCENCE ANALYSIS

Many drugs fluoresce when their powder is exposed to ultraviolet radiation. It is important to observe all materials on reaction with different chemical reagents under U.V. light. The fluorescence characteristics of powdered drug were studied under U.V. light after treating with different chemical reagents is reported.

Analysis of Extracts

The petroleum ether, chloroform, acetone, alcohol, aqueous extracts and drug powder of *Sida spinosa* as such were subjected to fluorescence analysis in day light and UV - light with different solvents like 1N sodium hydroxide (aqueous), 1N sodium hydroxide (alcoholic), 1N hydrochloric acid, 50% sulphuric acid, 50% nitric acid and methanol.

The results are given in **Table 3**

Table - 3
Fluorescence Analysis of Extracts and Drug powder of *Sida spinosa* Linn.

Reagents	Petroleum ether Extract		Chloroform Extract		Acetone Extract		Alcohol Extract		Aqueous Extract		Drug Powder	
	DL	UVL	DL	UVL	DL	UVL	DL	UVL	DL	UVL	DL	UVL
1 N NaOH (Alcohol)	Light green	Green	Green	Light green	Light green	Green	Green	Light green	Yellowish brown	Brown	Green	Green
1 N NaOH (Aqueous)	Light green	Green	Green	Green	Light green	Green	Green	Light green	Light brown	Brown	Pale green	Green
1 N HCl	Green	Blackish green	Dark green	Pale green	Green	Blackish green	Blackish green	Blackish green	Yellowish brown	Dark brown	Blackish green	Blackish green
50% H_2SO_4	Green	Blackish green	Green	Blackish green	Yellowish green	Blackish green	Blackish green	Blackish green	Yellowish brown	Dark brown	Blackish green	Blackish green
50% HNO_3	Light green	Light green	Light green	Dark green	Yellowish green	Green	Light green	Green	Brown	Light brown	Green	Light green
Methanol	Green	Green	Green	Green	Green	Light green	Green	Green	Brown	Light brown	Green	Green
Sample + NH_3 Solution	Green	Light green	Green	Light green	Green	Light green	Light green	Green	Light brown	Light brown	Light green	Light green
Sample + Iodine	Yellowish green	Dark green	Dark yellow	Light green	Light yellow	Light brown	Pale yellow	Light green	Blackish brown	Blackish brown	Light green	Dark green
Sample + $FeCl_3$	Pale green	Green	Light yellow	Green	Greenish yellow	Light brown	Pale yellow	Light brown	Light brown	Light brown	Green	Green

DL - Day Light **UVL - UV Light**

Elemental Analysis (Chaudhry, B.L., 2004)

Elemental Analysis is a gradual study process of given facts from materials like compounds, soil, inorganics, drinking or waste water and body fluids that are carefully scrutinized or observed for their elemental components or even to learn and analyze their isotopic components.

There are 2 varieties of Elemental Analysis namely the quantitative data and the qualitative data. Quantitative data pertains to the total number of elements present while the qualitative specific tells which elements are present.

The therapeutic powers of elements were recognized in Traditional Systems of Indian and Chinese Medicine. In recent years, health care scientists and nutritionist have realized significant benefits of elements as zinc, copper and iron in human health.

The adequate uptake of trace elements depends both on the amount and bioavailability in the diet consumed. The prevalence of trace element deficiencies is predicted to be higher in populations dependent on cereals as the staple food.

Estimation of Calcium

- The amount of calcium present in the test solution was determined by titrating the test solution against EDTA using murexide indicator in sodium hydroxide medium (2M).

Estimation of Sodium and Potassium by Flame Photometer

- These elements were estimated using flame photometer. The diluted extracts were automized in a calibrated flame photometer with the wavelength dial at 589 nm for sodium and 768 nm for potassium.

Estimation of Iron, Zinc, Manganese and Copper

- These elements were estimated in Atomic Absorption Spectrophotometer (AAS). The AAS has been calibrated to read different concentrations of zinc, iron, copper and manganese.

The concentration of elements determined was found to vary depending on the composition of the herbs used. Although the trend linking the element of the medicinal plants to its curative abilities could not be clearly determined, this study showed that the toxic elements found in the samples were below the levels prescribed by health regulations. Nevertheless, such data are important to understand the pharmacological action and the exact mechanisms of action and formation of active constituents for each medicinal plant and to decide the dosage of the herbs used in the final formulation.

The results for elemental analysis are shown in **Table 4**

Table - 4

Elemental Analysis of Whole Plant Powder of *Sida spinosa* Linn.

S.No	Name of the Parameter	Content
1	Organic Carbon (%)	15.1
2	Total Nitrogen (%)	2.10
3	Total Phosphorus (%)	0.60
4	Total Potassium (%)	2.50
5	Total Sodium (%)	0.30
6	Total Calcium (%)	1.45
7	Total Magnesiun (%)	0.21
8	Total Sulphur (%)	12.56
9	Total Zinc (ppm)	7.23
10	Total Copper (ppm)	16.9
11	Total Iron (ppm)	127.42
12	Total Manganese (ppm)	20.27
13	Total Boron (ppm)	10.23
14	Total Molybdenum (ppm)	0.021
15	Heavy Metals	Nil

PHYTOCHEMICAL INVESTIGATIONS

Plant Drying & Size Reduction

The whole plant of *Sida spinosa* was cut into small pieces and dried in shade 20 – 25^{o} C at room temperature. The plant material stored in PVC bag and stored in dark and clean place in airtight container.

The Phytochemical Investigations of a plant involve the following:

- ❖ Extraction of Plant material
- ❖ Identification of the Phytoconstituents
- ❖ Separation and Isolation of Phytoconstituents
- ❖ Characterization of the isolated Phytocontituents

Extraction

Extraction involves the separation of bioactive portion of the plant tissues from the inactive components by using selective solvents in standard extraction procedure. The products so obtained are relatively impure liquids, semisolids or powders. These include classes of preparation known as decoction, fluid extraction, tinctures, and powder **(Bhatnagar, S.P., *et al*., 2010).**

Extraction is carried out for following variety of reasons **(Saharan, *et al*., 2001).**

- To get the concentrate of active substance
- To purify the compounds for subsequent to processing
- To isolate the compound for characterization

Method

About 1 kg of the air dried powdered plant material was extracted successively with solvents of increasing polarity using soxhlet extractor.

❖ **Extraction of Plant material**

The plant material was dried in the shade, then the shade dried plant material was subjected to get coarse powder and it was extracted in soxhlet apparatus using various solvents according to their polarity

- Petroleum ether extract
- Chloroform extract
- Acetone extract
- Ethanolic extract
- Aqueous extract

Materials Required

Shade dried coarse powder of *Sida spinosa,* Petroleum ether, Chloroform, Acetone, Ethanol and 0.25% Chloroform water.

Preparation of Extracts

Petroleum ether Extract

The coarse powder of *Sida spinosa* was extracted with 2 - 3 litres of petroleum ether (60°- 80°C) by continuous hot percolation using soxhlet apparatus. After completion of extraction it was filtered and the solvent was removed by distillation under the reduced pressure. The extract was stored in desiccator.

Chloroform Extract

The marc left after extraction was dried and extracted with 2 - 3 litres of chloroform (55.5° - 61.5°C) by continuous hot percolation using soxhlet apparatus. After completion of extraction it was filtered and the solvent was removed by distillation under the reduced pressure. The extract was stored in desiccator.

Acetone Extract

The marc left after extraction was dried and extracted with 2 - 3 litres of acetone (55.5°C - 56.5°C) by continuous hot percolation using soxhlet apparatus. After completion of extraction it was filtered and the solvent was removed by distillation under the reduced pressure. The extract was stored in desiccator.

Ethanolic Extract

The marc left after acetone extraction was dried and extracted with 2 - 3 litres of ethanol 95^{o}C by continuous hot percolation using soxhlet apparatus. After completion of extraction, it was filtered and the solvent was removed by distillation under pressure. The extract was stored in a desiccator.

Aqueous Extract

The marc left after extraction was dried and extracted with 2 - 3 litres of Chloroform water (0.25%) by continuous hot percolation using soxhlet apparatus. After completion of extraction it was filtered and the solvent was removed by distillation under reduced pressure. The extract was stored in desiccator.

The results are shown in **Table 5**

Table - 5

Successive Solvent Extraction of *Sida spinosa* Linn.

Method of Extraction	**Extracts**	**Colour & Consistency**	**Average Extractive Value (% w/w)**
Continuous hot Percolation by Soxhlet Apparatus	Petroleum ether	Dark green	1.16
	Chloroform	Green	0.76
	Acetone	Light green	1.25
	Alcohol	Brownish green	5.61
Cold Maceration	Aqueous	Brown	8.52

All the above extracts used for

- Identification of constituents by phytochemical test
- Separation and isolation of plant constituents by chromatographic method
- Pharmacological studies

Phytochemical Evaluation (Kokate, C.K. *et al*., 1994)

The extracts were subjected to qualitative tests for the identification of the phytoconstituents present in it viz. alkaloids, carbhohydrates, glycosides, phytosterols, fixed oils & fats, phenolic compounds & tannins, proteins & free amino acids, gums & mucilages, flavonoids, lignins and saponins.

Test for Alkaloids

A small portion of the solvent free petroleum ether, chloroform, acetone, ethanol and aqueous extracts were stirred separately with few drops of dilute hydrochloric acid and filtered. The filtrate was tested carefully with various alkaloidal reagents such as

a. Mayer's reagent	-	Cream Precipitate
b. Dragendroff's reagent	-	Orange brown precipitate
c. Hager's reagent	-	Yellow precipitate
d. Wagner's reagent	-	Reddish brown precipitate

Test for Carbohydrates and Glycosides

The minimum amount of extracts were dissolved in 5ml of distilled water and filtered. The filtrate was subjected to test for carbohydrates and glycosides.

A. Molisch's test

The filtrate was treated with 2 - 3 drops of 1% alcoholic alpha - napthol and 2ml of concentrated sulphuric acid was added along the sides of the test tube.

B. Fehling's test

The filtrate was treated with 1ml of Fehling's solution and heated. Orange precipitate was obtained showing the presence of carbohydrates.

Another portion of the extract was hydrolysed with hydrochloric acid for few hours on a water bath and the hydrolysate was subjected to Legals, Borntrager's test to detect the presence of different glycosides.

Test for Flavonoids

A. With aqueous sodium hydroxide solution, blue to violet colour (antho cyanins), yellow colour (flavones), yellow to orange (flavonones).

B. With concentrated sulphuric acid, yellowish orange colour (Anthocyanins), yellow to orange colour (Flavones), orange to crimson (Flavonones).

C. Shinoda's Test

The extract was dissolved in alcohol, to it piece of magnesium turnings followed by Concentrate Hydrochloric acid drop wise were added and heated. Appearance of magenta colour showed the presence of flavonoids.

Test for Phytosterol

Libermann's Burchard Test

One gram of the extract was dissolved in few drops of acetic acid, 3ml of acetic anhydride was added followed by few drops of conc. sulphuric acid. Appearance of bluish green colour showed the presene of phytosterol.

Test for Fixed oils and Fats

A small quantity of the various extracts was separately pressed between two filter papers. No oil stain was observed on the paper which indicated the absence of fixed oil.

Few drops of 0.5 N alcoholic potassium hydroxide were added to small quantity of various extracts along with a drop of phenolphthalein. The mixture was heated on a water bath for 1 – 2 hours. Absence of soap formation.

Test for Saponins

Small quantities of extracts were taken separately in water and shaken for few minutes, appearance of foam showed the presence of saponins.

Test for Tannins and Phenolic Compounds

Small quantities of extracts were taken separately in water and tested for the presence of phenolic compounds and tannins with

i. Dilute ferric chloride solution - violet colour
ii. 1% solution of gelatin containing 10% NaCl - white precipitate
iii. 10% lead acetate solution - white precipitate

Test for Lignin

With alcoholic solution, phloroglucinol and hydrochloric acid, appearance of red colour showed the presence of lignin.

Test for Proteins and Free Amino Acids

Dissolve small quantities of extracts in a few ml of water and treated with,

i. Millon's reagent - Appearernce of red colour showed the presence of proteins and free amino acids.

ii. Ninhydrin reagent - Appearance of purple colour showed the presence of proteins and free amino acids.

iii. Biuret test - Equal volume of 5% solution of Sodium hydroxide and 1% solution of Copper sulphate were added. Appearance of pink colour showed the presence of proteins and free amino acids.

Test for Gums and Mucilages

About 10 ml of extract was added separately to 25 ml of absolute alcohol with constant stirring and filtered. The precipitate was dried in air and examined for its swelling properties and for the presence of carbohydrates.

The results of preliminary phytochemical screening of whole plant powder and extracts are shown in **Table 6**

Table - 6
Data Showing the Preliminary Phytochemical Screening of *Sida spinosa* Linn.

Phytoconstituents	Petroleum ether extract	Chloroform extract	Acetone extract	Ethanolic extract	Aqueous extract
Alkaloids	(-)	(+)	(+)	(+)	(-)
Carbohydrates	(-)	(+)	(+)	(+)	(-)
Glycosides	(-)	(-)	(-)	(+)	(-)
Flavonoids	(-)	(-)	(-)	(+)	(-)
Phytosterols	(+)	(+)	(-)	(+)	(-)
Fixed oils and Fats	(+)	(-)	(-)	(-)	(-)
Saponins	(-)	(-)	(+)	(+)	(+)
Phenolic compounds and Tannins	(+)	(+)	(+)	(+)	(+)
Lignins	(+)	(+)	(+)	(+)	(+)
Proteins and Free Amino acids	(-)	(+)	(-)	(-)	(-)
Gums and Mucilage	(+)	(-)	(-)	(+)	(+)

(+) Presence **(-) Absence**

Separation and Isolation of Plant Constituents by Chromatographic Methods

Of the various methods of separating and isolating the plant constituents, the chromatographic procedure originated by Tswett is one of the most useful technique for general application. All finely divided solids have the power to adsorb other substance on their surface to a greater or lesser extent, similarly, all substance are capable of being adsorbed, some much more readily than others. This phenomenon of selective adsorption is the fundamental principle of chromatography **(Egonsthal, *et al*., 2005; Markham, K.R., 1982).** In the present study Thin Layer Chromatography (TLC) and Column Chromatography methods were used.

Thin Layer Chromatography

Thin Layer Chromatography is so widely used that it has become an essential technique for analyst and research workers. TLC is the almost universal analytical technique in chemical analysis for organic and inorganic matter.

TLC is a simple and rapid method carried out using thin layer of adsorbents on plates. TLC not only combines the advantage of paper and column chromatography but in certain aspects it is found to be superior to either methods.

TLC is an important tool in the separation, identification and estimation of different classes of natural products. When a mixture containing different component is made to ascend in a TLC plate with the help of a solvent which acts as mobile phase, there will be a preferential adsorption of different compounds at different places on the plate. The result is the separation of components **(Biren, N. *et al*., 2008).**

Preparation of TLC Plate

80 g of silica gel G was weighed and shaken to a homogenous suspension with 85 ml of distilled water for 90 seconds. This suspension was poured in TLC applicator which was adjusted to 0.25 mm thickness. 20 carrier plate (20 x 5 cm) were laid together in a row on a template for air drying until the transparence of layer disappeared. The plates were dried in hot air oven at 110^{o} C for 30 minutes (activation). The plates were then stored in a dry atmosphere and used whenever required **(Kokate, C.K., *et al.*, 1994).**

Application of Extracts for Separation

The various diluted extracts spotted on a TLC plate 2 cm above its bottom using capillary tube. Most solution for application were between 0.1 – 1 % strength. The starting point was equally sized as far as possible and sports had diameter ranging from 2 – 5 mm.

$$R_f \text{ value} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

From the preliminary phytochemical screening, it was observed that more number of phytoconstituents were present in the ethanolic extract of *Sida spinosa*. The ethanolic extract of whole plant powder of *Sida spinosa* was subjected to Thin Layer Chromatography using different mobile phase that are suitable for detecting various phytoconstituents like alkaloids, glycosides, flavonoids, steroids, and essential oil **(Harborne, J.B., 2007). Fig – 21.**

Chromatograms for the ethanolic extracts were carried out using the procedure recommended by Indian Herbal Pharmacopoeia and the R_f value of the ethanolic extract are tabulated in **Table – 7.**

Table - 7

Thin Layer Chromatography of Ethanolic Extract of *Sida spinosa* Linn.

S.No	Mobile Phase	Detecting Agent	No. of Spots	R_f Value
1	Benzene : Ethanol (9 : 1)	Dragendroff reagent	3	0.57 0.67 0.72
2	Toluene : Ethyl acetate (7 : 3)	Anisaldehyde sulphuric acid	1	0.61
3	Petroleum ether : Ethyl acetate (2 : 1)	Iodine vapour	2	0.75 0.71
4	Benzene : Ethyl acetate (9 : 1)	Vanillin in sulphuric acid	3	0.54 0.66 0.77
5	Chloroform	Vanillin in sulphuric acid	1	0.65

Column Chromatography (Mabry, T.J., 1970; Markham, R., 1982; Clark, 1986)

Column chromatography is a method used to purify individual chemical compounds from mixtures of compounds. It is often used for preparative application on scales from micrograms upto kilograms.

Column chromatography is suitable for the physical separation of gram quantities of material. A solvent act as the mobile phase while a finely divided solid surface act as the stationary phase. The stationary phase will adsorb the components of the mixture to varying degrees. As the solution containing mixture passes over the adsorbent surface. This process may be described by three-way equilibrium between the sample, the solvent and the adsorbent.

The solvent and sample compete for positions on the solid adsorbent, the solvent displacing the sample reversibly and continuously in the direction of the solvent, and will spend more time in the solvent, and will therefore be eluted first.

Preparation of Column (Raymond P.W., 2002)

Material used: Column of size 90 x 2.5 cm

Silica gel (100 – 200 mesh) as an adsorbent

The ethanolic extract of *Sida spinosa* were subjected to Column Chromatography over silica gel (100 - 200 mesh). The column was eluted with solvents of increasing polarity. They were

Solvents used for Column Chromatography

1. Petroleum ether 100%
2. 80 % Petroleum ether and 20% Chloroform
3. 60% Petroleum ether and 40% Chloroform
4. 50% Petroleum ether and 50% Chloroform
5. 40% Petroleum ether and 60% Chloroform
6. 20% Petroleum ether and 80% Chloroform
7. Chloroform 100%
8. 80% Chloroform and 20 % Acetone
9. 60% Chloroform and 40% Acetone
10. 50% Chloroform and 50% Acetone
11. 40% Chloroform and 60% Acetone
12. 20% Chloroform and 80% Acetone
13. Acetone 100%
14. 80% Acetone and 20% Ethylacetate
15. 60% Acetone and 40% Ethylacetate
16. 50% Acetone and 50% Ethylacetate
17. 40% Acetone and 60% Ethylacetate
18. 20% Acetone and 80% Ethylacetate
19. Ethylacetate 100%
20. 80% Ethylacetate and 20% Methanol
21. 60% Ethylacetate and 40% Methanol
22. 50% Ethylacetate and 50% Methanol
23. 40% Ethylacetate and 60% Methanol
24. 20% Ethylacetate and 80% Methanol
25. Methanol 100%

Method

The Ethanolic extract of *Sida spinosa* was added to the top of the column, separately by dissolving in a minimum amount of the solvent used to pack the column. The sample added carefully down the side of the column. Solvent drawn from the bottom of the column and fresh solvent was carefully added to the column, so that the solvent was continuously flowing through the column. Fractions of a standard volume were collected. The identities of the fraction were determined by Thin Layer Chromatography.

As per Ethanomedical information Ethanolic extract of *Sida spinosa* Linn. has been claimed to possess Hypoglycemic activity and it showed characteristic spots in TLC. Based on these considerations Ethanolic extract of *Sida spinosa* Linn. was subjected to Column Chromatography over silica gel (60 – 120 mesh). The column was eluted with solvents of increasing polarity. They are

The results are shown in **Table 8**

Table – 8

Column Chromatography of Ethanolic Extract of *Sida spinosa*

S.No	Eluent	Ratio	Nature of Residue	Compound
1	Petroleum ether	100	No residue	-
2	Petroleum ether: chloroform	80:20	No residue	-
3	Petroleum ether: Chloroform	60:40	No residue	-
4	Petroleum ether: Chloroform	50:50	No residue	-
5	Petroleum ether: Chloroform	40:60	No residue	-
6	Petroleum ether: Chloroform	20:80	No residue	-
7	Chloroform	100	Green residue	-
8	Chloroform: Acetone	80:20	Yellow residue	-
9	Chloroform: Acetone	60:40	Yellow residue	-
10	Chloroform: Acetone	50:50	No residue	-
11	Chloroform: Acetone	40:60	No residue	-
12	Chloroform: Acetone	20:80	No residue	-
13	Acetone	100	Pale green residue	-
14	Acetone: Ethylacetate	80:20	Yellow residue	-
15	Acetone: Ethylacetate	60:40	Yellow residue	-
16	Acetone: Ethylacetate	50:50	Yellow crystals	SS1
17	Acetone:Ethylacetate	40:60	Yellow residue	-
18	Acetone:Ethylacetate	20:80	No residue	-
19	Ethylacetate	100	No residue	-
20	Ethylacetate: Methanol	80:20	No residue	-
21	Ethylacetate: Methanol	60:40	No residue	-
22	Ethylacetate: Methanol	50:50	No residue	-
23	Ethylacetate: Methanol	40:60	Brown residue	-
24	Ethylacetate: Methanol	20:80	Brown residue	-
25	Methanol	100	Brown residue	-

An yellow crystalline compound was obtained by column chromatography in the fractions of Ethanolic extract (Acetone : Ethyl acetate 50 : 50) and was named SS1.

Description of the Isolated Compound SS1

Nature	:	Crystalline
Colour	:	Yellow
Taste	:	Bitter
Solubility	:	Soluble in Methanol and Ethyl acetate
Melting Point	:	155° - 160°c
TLC	:	Solvent System Chloroform: Ethyl Acetate: Methanol: Water (15 : 40 : 22 : 5.0)
Identification Test	:	Positive for Libermann's Burchard Test (Phytosterol)

Spectroscopy (Sharma, Y.R., 1980; Silverstein, 2004; Beckett, A.H., and Stanlake, J.B., 2005)

Spectroscopy is the measurement and interpretation of Electro Magnetic Radiation **(EMR)** absorbed or emitted when the molecules or atoms or ions of a sample move from one energy state to another energy state.

The arrangements of all types of electromagnetic radiations in order of their increasing wave lengths or decreasing frequencies is known as complete electromagnetic spectrum.

Visible and Ultra Violet radiations cover the wavelength range from 200 – 800nm.The absorption of radiation in this region cause the excitation of π electron conjugated or an unconjugated system. In case of a conjugated system, the separation between the ground state and the excited energy level will be less and hence absorption occours at a longer wavelength. Also carbonyl group of an aldehyde or a ketone absorbs at some characteristic wavelengths. Thus an ultraviolet or visible spectrum is quite useful for the detection of conjugation, carbonyl group etc. and may not provide any information about the remaining part of the molecule.

The different types of EMR are visible radiation, UV radiation, IR radiation, Microwaves, Radio waves, X rays, Gamma rays, or Cosmic rays. as these radiations have different wavelength or frequency or energy.

Infra Red Spectroscopy (IR)

Infra Red spectrum is an important record which gives sufficient information about the structure of a compound. The absorption of Infra Red radiation causes the various bands in a molecule to stretch and bend with respective one another. The most important region for an organic chemist is 2.5 μ to 15 μ in which molecular vibrations can be detected and measured in an Infra Red region.

Infra Red spectroscopy offers the possibility to measure different types of atomic bond vibrations at different frequencies. Especially in organic chemistry the analysis of IR absorption spectra shows what type of bonds is present in the sample. It is also an important method for analyzing polymers and constituents like filters, pigments and plasticizers.

Identification of functional group and structure elucidation, the entire IR region is divided into

- Group frequency region : 4000 – 1500 cm^{-1}
- Finger print region : 1500 – 400 cm^{-1}

In the frequency region, the peaks corresponding to different functional groups can be observed. (eg) Amino group, Alcoholic group etc.

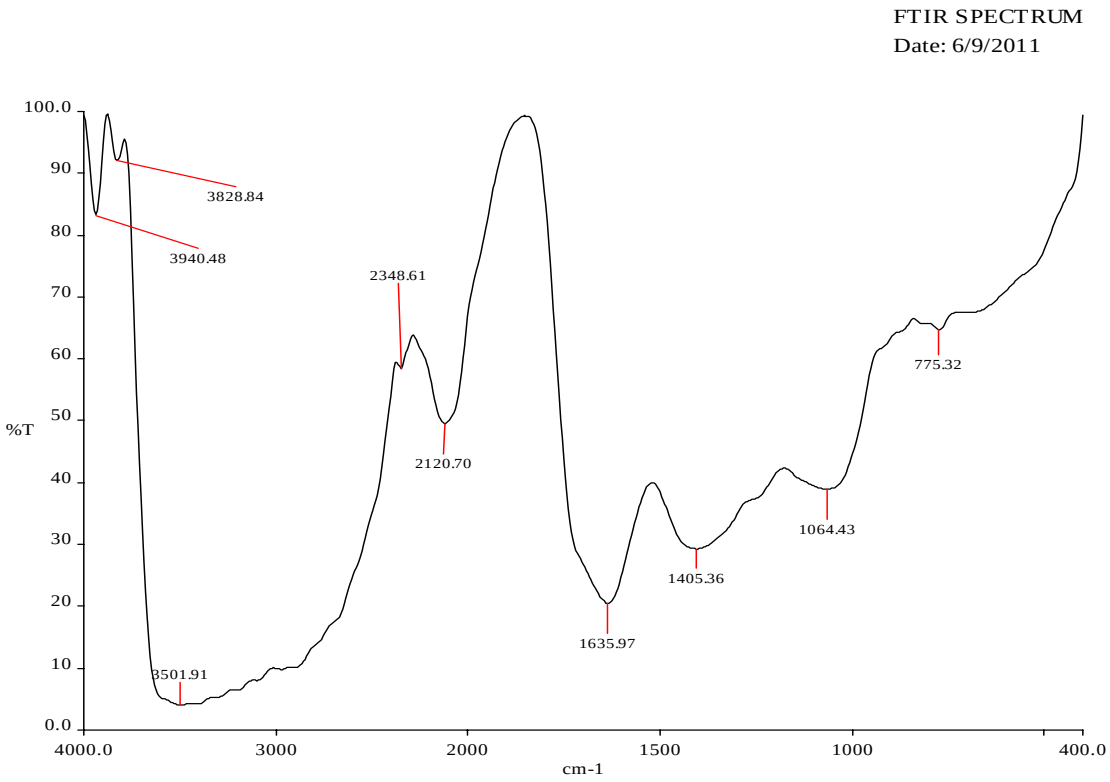
The crystals obtained from ethanolic extract of *Sida spinosa* were subjected to Infrared Spectroscopy and the spectrum is shown in **Fig – 22.**

IR Spectra of the crystalline compound SS1 showed characteristic absorption (in cm^{-1}) at

- 3828 – 3940 - O – H Stretching
- 3501 - N – H Stretching
- 3000 - O – H Stretching
- 2348.61 - COOH and Enols
- 2120.70 - C ≡ N Nitrites
- 1635.97 - Primary amines
- 1405 - O – H Bending
- 1064 - C – O Stretching
- 775.32 - N – H Wagging

Fig - 22

IR Spectrum of SS1



000.00 400.00 4.01 100.00 4.00 %T 5 1.00

REF 4000 99.48 2000 68.20 600
3940.48 83.26 3828.84 92.06 3501.91 4.01 2348.61 58.45 2120.70 49.54
1635.97 20.44 1405.36 29.27 1064.43 39.01 775.32 64.76

High Performance Thin Layer Chromatography (HPTLC)

The High Performance Thin Layar Chromatography is a method of separation in which the stationary phase is contained in a column, one end of which is attached to a source of pressurized liquid eluent (mobile phase)

- Instrument : Developed by Kirkland and Huber
- Pressure : $2.07 * 10^7$ Nm^{-2} (3000 psi)
- Diameter of column : 1 - 3 mm

High Performance Thin Layer Chromatography finds many applications, including

- Radiochemical purity of radiopharmaceuticals
- Determination of the pigments in plant.
- Detection of pesticides or insecticides in food
- Analyzing the dye composition of fibres in forensics
- Identifying compounds present in a given substance
- Monitoring organic reactions

The crystals obtained from ethanolic extracts of *Sida spinosa* were subjected to HPTLC in different concentrations, and the HPTLC profile are shown in **Fig**. **23 - 25** and **Table 10 – 12**

CHROMATOGRAPHIC CONDITION FOR HPTLC FINGER PRINT

Sample Name	:	SS1
Solubility	:	Ethanol
Stationary phase	:	Silica gel 60 F 254
Mobile phase	:	Chloroform: Ethyl Acetate: Methanol: Water (15 : 40 : 22 : 5.0)
Scanning wavelength	:	298 nm
Sample concentration	:	20 mg/ml
Applied volume	:	5.0, 10 & 20 µl
Development mode	:	Ascending mode

Fig - 25

HPTLC PROFILE OF SS1

5.0 µl Concentration of SS1

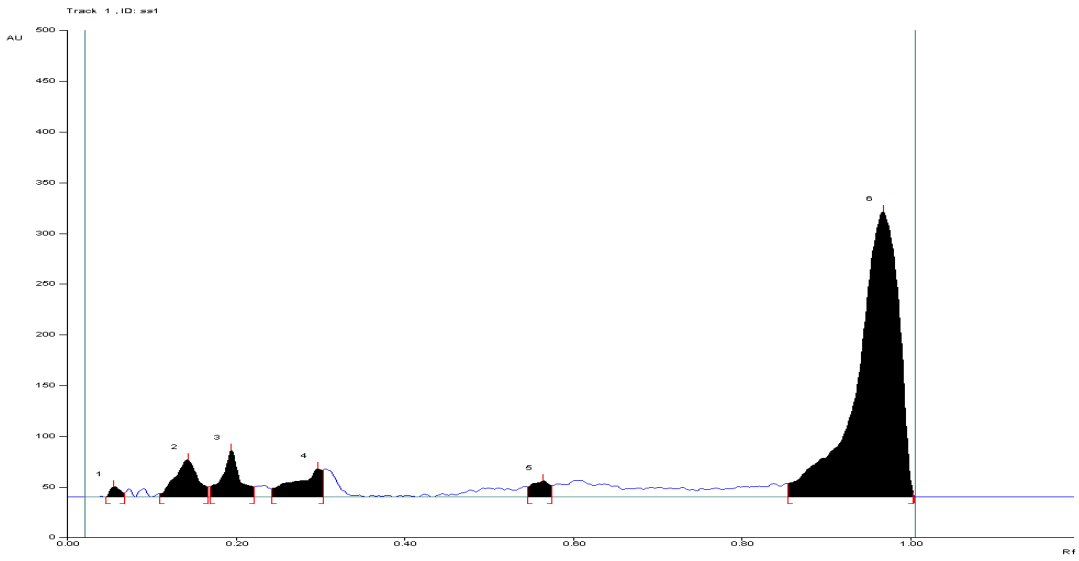


Table – 9

HPTLC Profile of SS1 (5 µl)

Peak	Start R_f	Start Height	Maximum R_f	Maximum Height	Maximum %	End R_f	End Height	Area	Area %
1	0.05	0.2	0.05	10.3	2.46	0.07	3.9	86.2	0.75
2	0.11	3.3	0.14	36.7	8.79	0.17	9.8	664.0	5.75
3	0.17	10.2	0.19	45.9	10.99	0.22	10.2	635.7	5.51
4	0.24	8.2	0.30	27.9	6.69	0.30	26.8	611.5	5.30
5	0.55	9.7	0.56	15.7	3.76	0.58	11.3	235.2	2.04
6	0.85	13.3	0.97	281.2	67.31	1.00	3.8	9311.9	80.66

Fig - 26

HPTLC PROFILE OF SS1

10.0 µl Concentration of SS1

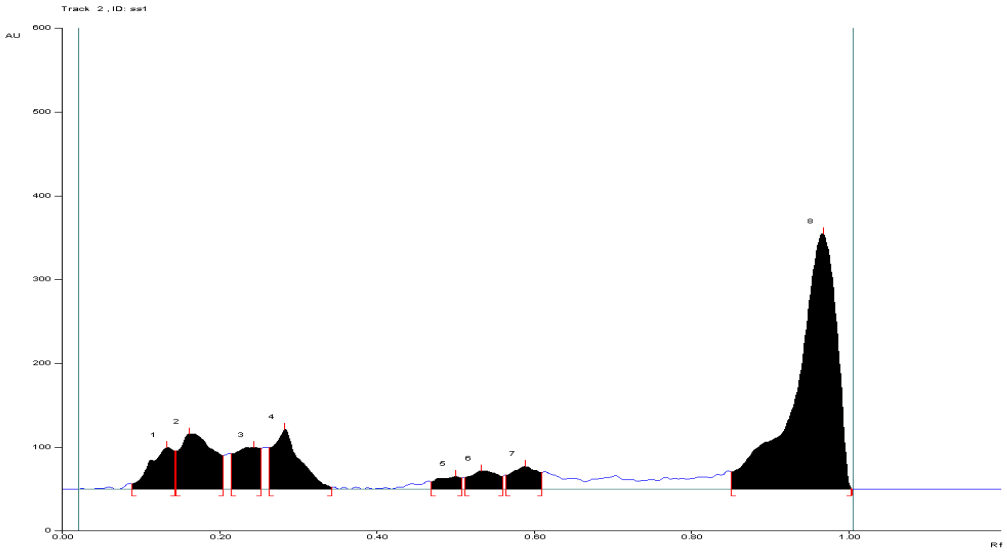


Table – 10

HPTLC Profile of SS1 (10 µl)

Peak	Start R_f	Start Height	Maximum R_f	Maximum Height	Maximum %	End R_f	End Height	Area	Area %
1	0.09	6.6	0.13	49.5	8.17	0.14	45.5	1019.9	5.77
2	0.15	45.6	0.16	66.1	10.91	0.20	39.8	1938.8	10.98
3	0.22	42.4	0.24	50.1	8.27	0.25	48.9	1093.0	6.19
4	0.26	49.8	0.28	71.2	11.75	0.34	2.20	1654.6	9.37
5	0.47	8.70	0.50	15.2	2.51	0.51	13.9	306.4	1.74
6	0.51	14.0	0.53	21.8	3.59	0.56	15.6	533.3	3.02
7	0.56	17.0	0.59	27.0	4.45	0.61	20.2	607.7	3.44
8	0.85	20.7	0.97	304.9	50.34	1.00	1.10	10498.1	59.48

Fig - 27

HPTLC PROFILE OF SS1

20.0 µl Concentration of SS1

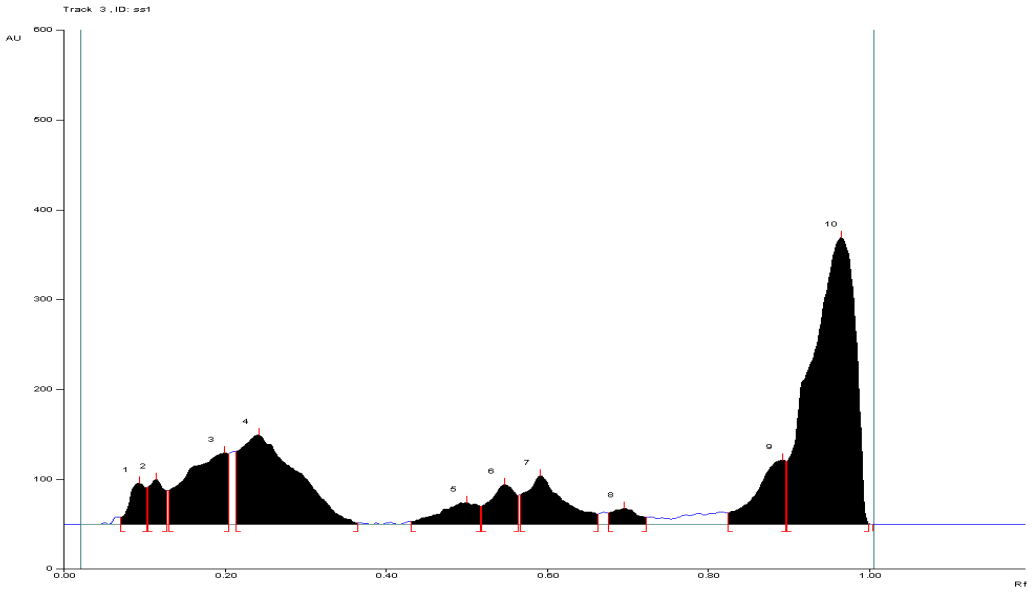


Table – 11

HPTLC Profile of SS1 (20 µl)

Peak	Start R_f	Start Height	Maximum R_f	Maximum Height	Maximum %	End R_f	End Height	Area	Area %
1	0.07	8.00	0.09	46.0	5.70	0.10	40.9	619.5	2.37
2	0.10	41.4	0.11	49.8	6.18	0.13	37.5	655.2	2.51
3	0.13	37.7	0.20	79.6	9.87	0.20	78.7	2801.5	10.73
4	0.21	81.3	0.24	99.5	12.35	0.37	1.40	4857.9	18.61
5	0.43	2.90	0.50	24.2	3.00	0.52	20.0	768.0	2.94
6	0.52	20.1	0.55	44.2	5.49	0.56	32.3	942.9	3.61
7	0.57	32.7	0.59	54.0	6.70	0.66	11.2	1721.2	6.59
8	0.68	12.9	0.70	17.6	2.19	0.72	7.80	392.2	1.50
9	0.82	12.5	0.89	71.4	8.86	0.90	70.7	1713.7	6.56
10	0.90	70.0	0.97	319.5	39.66	1.00	0.40	11635.7	44.57

Gas Chromatography – Mass spectroscopy (GC–MS)

In the simplest terms the GC-MS instrument represents a device that separates chemical mixtures (the GC component) and a very sensitive detector (the MS component) with a data collector (the computer component).

Once the sample solution is introduced into the GC inlet it is vaporized immediately because of the high temperature (250 degrees C) and swept onto the column by the carrier gas (usually Helium).

The sample flows through the column experiencing the normal separation processes. As the various sample components emerge from the column opening, they flow into the capillary column interface. This device is the connection between the GC column and the MS. Some interfaces are separators and concentrate the sample via removal of the helium carrier.

The sample then enters the ionization chamber. Two potential methods exist for ion production. The most frequently used method in the Toxicology lab is electron impact (EI). The occasionally used alternative is chemical ionization (CI). For electron impact ionization a collimated beam of electrons impact the sample molecules causing the loss of an electron from the molecule. A molecule with one electron missing is represented by M+ and is called the molecular ion (or parent ion). When the resulting peak from this ion is seen in a mass spectrum, it gives the molecular weight of the compound. Chemical ionization begins with ionization of methane (or other gas), creating a radical which in turn will impact the sample molecule to produce M.H+ molecular ions.

Some of the molecular ions fragment into smaller daughter ions and neutral fragments. Both positive and negative ions are formed but only positively charged species will be detected.

Less fragmentation occurs with CI than with EI, hence CI yields less information about the detailed structure of a molecule, but does yield the molecular ion; sometimes the molecular ion cannot be detected by the EI method, hence the two methods complement one another. Once ionized, a small positive potential is used to repel the positive ions out of the ionization chamber.

The next component is a mass analyzer (filter), which separates the positively charged particles according to their mass. Several types of separating techniques exist; quadrupole filters, ion traps, magnetic deflection, time-of-flight, radio frequency, cyclotron resonance and focusing to name a few. The most common are quadrupoles and ion traps.

After the ions are separated according to their masses, they enter a detector and then on to an amplifier to boost the signal. The detector sends information to the computer which acts as a "clearing house". It records all the data produced, converts the electrical impulses into visual displays and hard copy displays. The computer also drives the mass spectrometer.

Identification of a compound based on it's mass spectrum relies on the fact that every compound has a unique fragmentation pattern. Even isomers can be differentiated by the experienced operator. Generally, more information is generated than could possibly be used. A library of known mass spectra which may be several thousand compounds in size is stored on the computer and may be searched using computer algorithms to assist the analyst in identifying the unknown.

It is important to incorporate all other available structural information (chemical, spectral, sample history) into the interpretation wherever appropriate.

The ultimate goal is accurate identification of a compound, which can be facilitated by the utilization of the GC-MS.

Instrument Details

Make	:	Perkin Elmer Clarus 500
Column Type	:	Capillary Column Elite-5 (5% Phenyl 95% dimethylpolysiloxane)
Column Length	:	30m
Column id	:	250µm

GC Conditions

Oven Program	:	50°C @ 6°C to 150°C (2min) @ 6°C to 290°C (5min)
Injector temp.	:	290°C
Carrier gas	:	He @ 1ml/min
Split ratio 1	:	10

MS Conditions

Mass Range	:	40 - 600 amu
Electron energy	:	70 ev
Source and Inlet line Temperature	:	200°C
Scan mode	:	Full Scan
Library used	:	NIST 2005
Sample injected	:	1µl

Chromatogram:

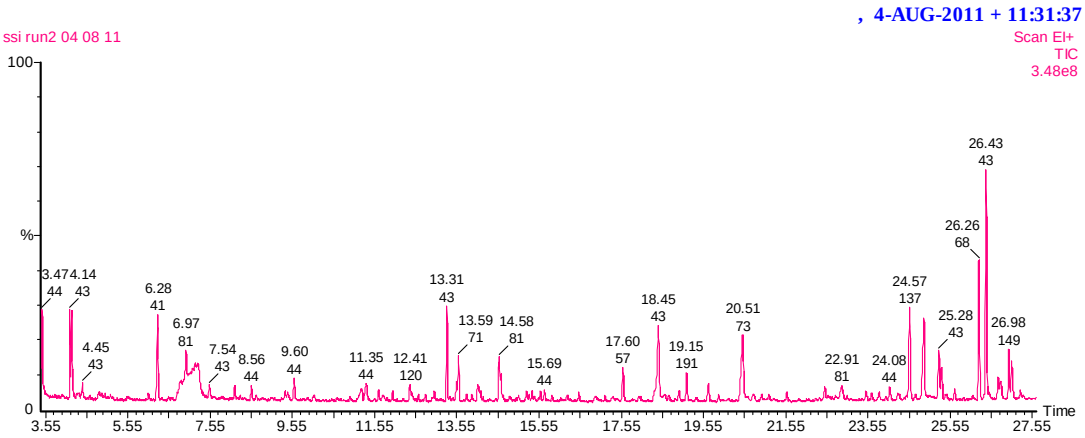


Fig – 28 Chromatogram of SS1 (a)

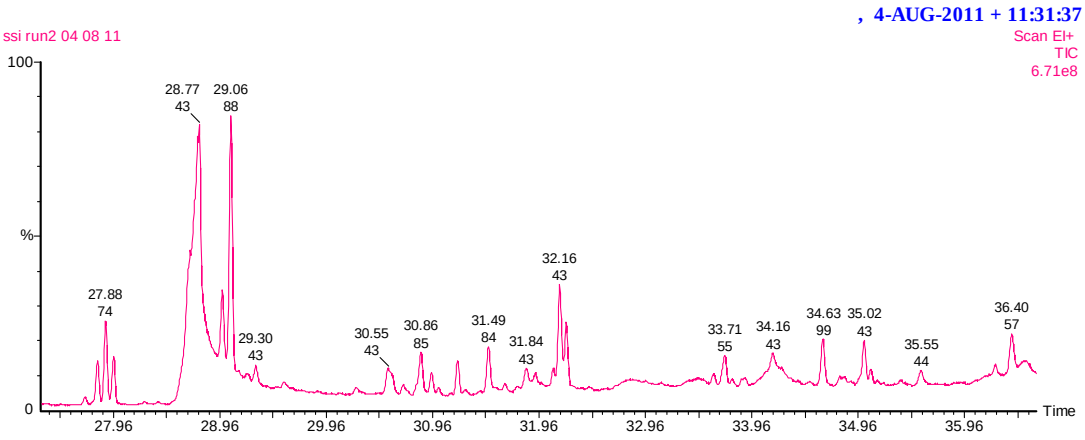


Fig – 29 Chromatogram of SS1 (b)

From the GC - MS Spectra of Isolated Compound SS1, the Following Structures are Proposed by Comparing with NIST 2005 Library

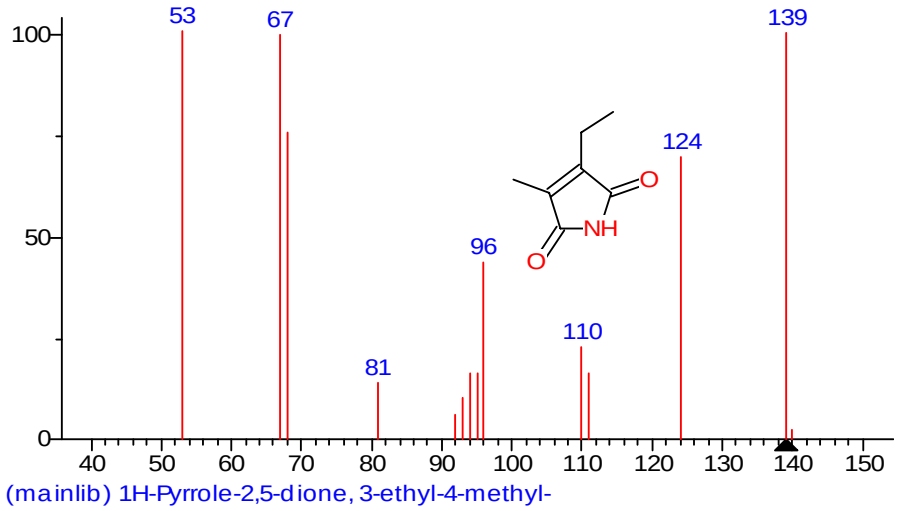


Fig – 30 GC - MS Spectra of Isolated Compound SS1 (a)

Formula	**-**	**$C_7H_9NO_2$**
Molecular Weight	**-**	**139**

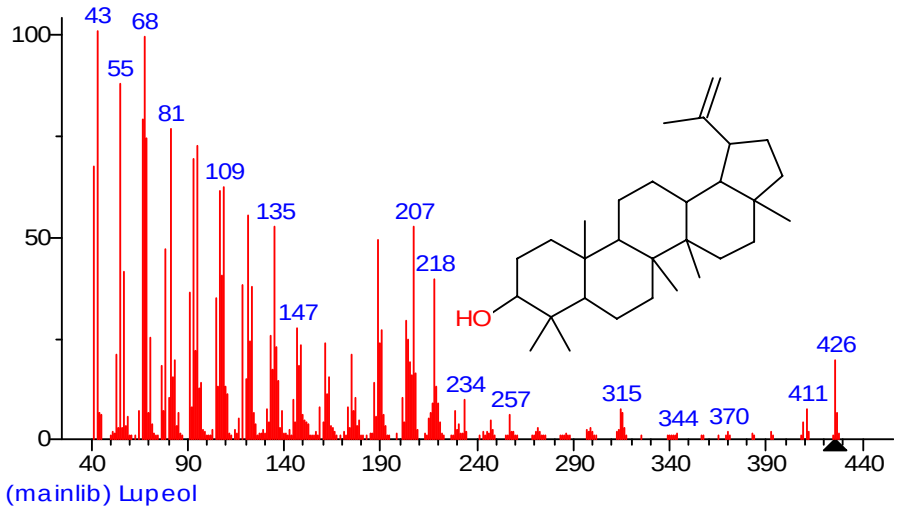


Fig – 31 GC - MS Spectra of Isolated Compound SS1 (b)

Formula	**-**	**$C_{30}H_{50}O$**
Molecular Weight	**-**	**426**

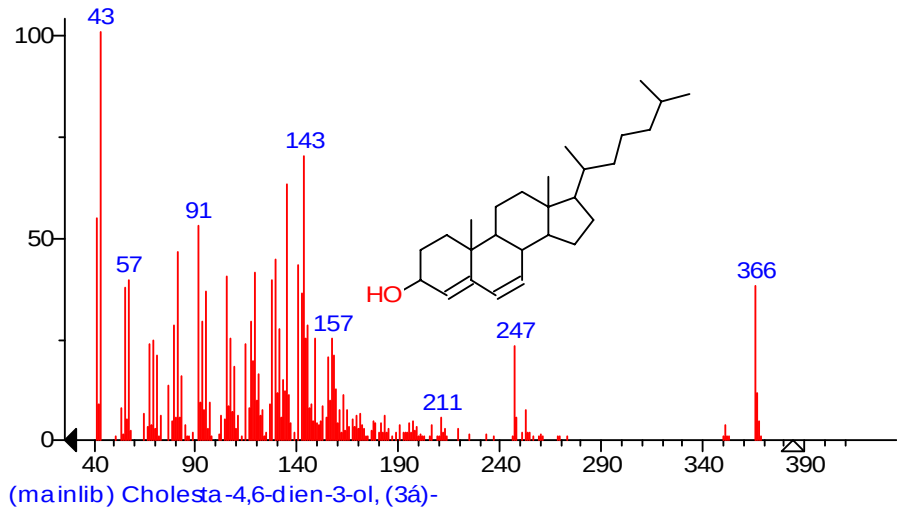


Fig – 33 GC - MS Spectra of Isolated Compound SS1 (c)

Formula	**-**	**$C_{27}H_{44}O$**
Molecular Weight	**-**	**384**

PHARMACOLOGICAL INVESTIGATIONS

Hypoglycemic Activity

Diabetes mellitus is a heterogeneous metabolic disorder as old as mankind and its incidence is considered to be high (4 - 5%) all over the world **(Pickup, J.C., *et al.*, 1997).** Diabetes is a chronic disorder of carbohydrate, fat and protein metabolism characterized by elevation of both fasting and post - prandial blood sugar levels. The synthetic oral hypoglycemic agents can produce serious side effects **(Akhtar, M.S., *et al.*, 1997, Holman, R.R., *et al.*, 1991).** In addition they are not considered safe for use during pregnancy **(Larner, J., *et al.*, 1985).** Furthermore, after the recommendation made on diabetes mellitus, investigation on hypoglycemic agents from medicinal plants have become important. Plants have played a major role in the introduction to new therapeutic agents. A multitude of herbs, spices and other plant materials have been described for the treatment of diabetes throughout the world **(Kesari, A.N., *et al.*, 2005).**

Medicinal Plants might provide a useful source of new oral hypoglycemic compounds for development of pharmaceutical entities or as a dietary adjunct to existing therapies **(Bailey, L.J., *et al.*, 1989).** Few of the plants used for the treatment of diabetes have received scientific or medical scrutiny and even the WHO expert committee on diabetes recommends that this area warrant further attention **(WHO, 1980).** Despite the presence of known antidiabetic medicines in the pharmaceutical market, screening for new antidiabetic sources from natural plants is still attractive because they contain substances that have an alternative and safe effect on diabetes mellitus.

Sida spinosa Linn. a rare medicinal plant reported to posses various medicinal properties and claimed to be used in the treatment of diabetes.

Acute Toxicity Study

Toxicity Evaluation in Mice: (OECD, 423 Guidance)

The Institutional Animal Ethical Committee (Sanction Reg. No. 265/CPCSEA/October 2000) approved the pharmacological protocols, according to prescribed guidelines of Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Acute toxicity of the ethanol extract was tested in mice. To determine acute toxicity of a single oral administration of the herbal drug, different doses of the drug (10, 100, 500, 1000, and 2000 mg/kg) were administered to different groups of mice (3 mice were used for each group, control mice received normal saline). Mortality and general behavior of the animals were observed periodically for 48 h. The animals were observed continuously for the initial 4 h and intermittently for the next 6 h and then again at 24 h and 48 h following drug administration. The parameters observed were grooming, hyperactivity, sedation, altered respiratory rate and convulsion.

Evaluation of Hypoglycemic Activity of Extracts (Vivek Kumar Sharma, *et al*., 2010)

The Hypoglycemic activities of ethanolic extract of *Sida spinosa* Linn. were studied by using Albino rats. Diabetes was induced using Alloxan.

Male Wistar Albino Rats (180 – 200 gm) were used for the study. Animals were fasted for 16 h but were allowed for free access to water prior to the experiment. Fasted rats were divided into five groups of six animals each.

Group I - Served as Control

Group II - Diabetic control (Alloxan 120 mg/kg/i.p)

Group III - Standard drug (Glibenclamide 10 mg/kg/p.o)

Group IV - Ethanolic Extract of *Sida spinosa* (200 mg/kg/p.o)

Group V - Ethanolic Extract of *Sida spinosa (*400 mg/kg/p.o)

Effect of Alloxan - Induced Diabetic Rats (Vivek Kumar Sharma, *et al*., 2010)

Male Wistar Albino Rats (180 – 200 gm) were made diabetic by single i.p injection of 120 mg/kg body weight of alloxan monohydrate (5% w/v in water) (Spectrochem Pvt. Ltd. Mumbai - India). After 4 days, blood samples were withdrawn and glucose levels were determined to confirm the development of diabetes (>350 mg/100 ml)

Serum Cholesterol (Bu-Chinu, *et al.*, 2003; Wang Chuan Chen, 2004)

Principle

This method is based Upon the principle that when cholesterol reacts with ferric percholate produce a lavender colour complex, in the presence of ethyl acetate and sulphuric acid when heated in a boiling water bath. The intensity of the colour produced is proportional to the cholesterol concentration.

Reagents

1. Cholesterol reagent - 250 ml
2. Precipitating reagent - 5 ml
3. Standard (200 mg%) - 3 ml

Technique

Assay of Cholesterol

Blood serum transferred into clean dry tubes labeled Blank (B), Standard (S), Test for Total Cholesterol (T). Then it was mixed well and immediately placed in a boiling water bath for exactly 60 seconds. Immediately cooled under running tap water and the Absorbance of Test (T) and Standard (S) against Blank (B) was measured on a photometer at 560 nm.

$$\text{Total Cholesterol in mg \%} = \frac{\text{A of (T)}}{\text{A of (S)}} \times 200$$

Table - 12

Reagent	B	S	T
Cholesterol	5.0 ml	5.0 ml	5.0 ml
Distilled water	0.025 ml	-	-
Standard	-	0.025 ml	-
Sample	-	-	0.025 ml

Triglycerides (Bu-Chinu, *et al.*, 2003; Wang Chuan Chen, 2004)

Principle

Triglycerides are hydrolysed by lipase to glycerol and free fatty acids. Glycerol is phosphorylated by ATP in the presence of Glycerol Kinase (GK) to Glycerol–3-Phosphate (G–3-P) which is oxidized by the enzyme Glycerol–3-Phosphate Oxidase (G–3-O) producing hydrogen peroxide. Hydrogen peroxide so formed reacts with 4–amino-antipyrine and 3, 5 Dichloro–2-Hydroxy Benzene Sulphonic acid (DHBS) in the presence of the enzyme peroxidase (POD) to produce a red quinine imine dye. The intensity of the colour developed is proportional to the triglycerides concentration.

Triglycerides + H_2O -------> Glycerol + Fatty acids.

GK

Glycerol + ATP ----------> G-3-P + ADP

GPO

G-3-P + O_2 ----------> H_2O + Dihydroxyacetone phosphate

POD

H_2O_2 + 4 – aminoantipyrine + DHBS -------> Red quinoneimine dye + H_2O

Reagents

1. Enzyme Reagent
2. Buffer solution
3. Triglycerides Standard 200 mg%

Technique

Three clean test tubes were labeled as Blank (B), Standard (S), Test (T). In each tube the solutions were pipetted out as shown below:

Table - 13

Reagents	(B)	(S)	(T)
Working enzyme Reagent	1.0 ml	1.0 ml	1.0 ml
Distilled water	0.01 ml	-	-
Triglycerides Standard	-	0.01 ml	-
Serum/Plasma	-	-	0.01 ml

It was mixed well and incubated at 37^o C for 10 minutes. The absorbance(A) of Standard(S) and Test(T) against Blank(B) was measured on a spectrophotometer at 510 nm.

Calculation

$$\text{Serum Triglyceride in mg \%} = \frac{\text{A of (T)}}{\text{A of (S)}} \times 200$$

Histopathology

A portion of pancrease tissue in each group was fixed in 10% Formaldehyde (Formalin diluted to 10% with normal saline) and proceeded for histopathology.

After paraffin embedding and block making, serial sections of 5µ thickness were made, stained with hematoxylin and eosin and examined under microscope. Photomicrographs of representative type were also taken.

The cellular integrating and architecture were intact in the control group. Pancreatic section stained with hematoxylin and eosin (H & E) showed that alloxan caused severe necrotic changes of pancreatic islets, especially in the centre of islets. Nuclear changes, karyolysis, disappearance of nucleus and in some places residue of destroyed cells were visible. Relative reduction of size and number of islets especially around the central vessel and severe reduction of beta cells were clearly seen in Diabetic control.

Study of pancreas of treated diabetic group showed increased size of islets and hyperchromic nucleus in section stained with H & E. There was also a relative increase of granulated and normal beta cells in the diabetic group which consumed 200mg/kg body weight of ethanolic extract, when compared with the diabetic group which consumed 400 mg/kg body weight of ethanolic extract of *Sida spinosa* Linn.

Pancreas of the diabetic group which consumed Glibenclamide 10 mg/kg body weight showed close similarity to group which consumed with test extract.

Statistical Analysis

Statistical Analysis was performed using one way analysis of varience (ANOVA) followed by Dunnett's-t. Test. Values expressed as mean ± SEM from six rats in each group. P – value < 0.05 were considered significant.

Table - 14

Effect of Ethanolic extract of *Sida spinosa* Linn. on Blood Glucose Level in Alloxan Induced Diabetic Rats

Treatment	Blood Glucose Level (BGL) in mg dL^{-1}			
	Initial	4th Day	7th day	10th Day
Control	85.67±1.585	87.00±1.693	86.33±2.155	83.00±4.405
Diabetic Control	298.8±5.205	322.0±5.568	358.3±3.774	354.0±8.359
Standard(Glibenclamide) (10 mg/kg)	305.8±4.929*	222.0±6.923*	175.7±4.522*	137.7±4.849*
Ethanolic Extract of *Sida spinosa* Linn. (200 mg/kg)	304.0±6.512*	229.3±5.766*	190.5±5.058*	190.7±2.813*
Ethanolic Extract of *Sida spinosa* Linn. (400 mg/kg)	306.3±6.249*	224.0±4.412*	200.3±5.920*	182.8±8.304*

Values are mean ± SEM; n = 6; *P < 0.05 Vs Diabetic Control

Table – 15

Effect of Ethanolic extract of *Sida spinosa* Linn. on Biochemical Parameters in Alloxan Induced Diabetic Rats

Treatment	Triglycerides	Total Cholesterol
Control	44.42 ± 0.595*	108.50 ± 4.332*
Diabetic Control	50.60 ± 1.569*	118.00 ± 6.361*
Standard (Glibenclamide) (10 mg/kg)	45.43 ± 0.869*	105.6 ± 2.932*
Ethanolic Extract of *Sida spinosa* Linn. (200 mg/kg)	47.60 ± 0.757*	116.6 ± 4.737*
Ethanolic Extract of *Sida spinosa* Linn. (400 mg/kg)	46.08 ± 1.511*	113.5 ± 5.506*

Values are mean ± SEM; n = 6; P < 0.05 Vs Diabetic Control

ANTIMICROBIAL ACTIVITY (Jeanne Moldenhauer, 2005)

Cruickshank, R. *et al.*, 1975; Mims, C, *et al.*, 1999; Murray, R. P., *et al.*, 2002)

Principle

Discs impregnated with known concentration of antibiotics are placed on an agar plate that has been inoculated uniformly over the entire plate with a culture of the bacterium to be tested. The plate is incubated for 18 to 24 hours at 37 ^{o}C (for bacterium). For fungi the plate is incubated for 24 to 48 hours at 25 ^{o}C. During this period, the antimicrobial agent diffuses through the agar, and may prevent the growth of the organism. Effectiveness of susceptibility is proportional to the diameter of the inhibition zone around the disc. Organisms which grow upto the edge of the disc are resistant.

Materials Required

- Whatmann No : 2 filter paper of 6mm
- Muller Hinton Agar plate (for Bacteria)
- Sabourand Dextrose Agar plate (for Fungi)
- Forceps
- Cotton swab
- Standardized inoculums
- Standard antibiotic disc

Preparation of Inoculum

The test micro organisms were obtained from National Chemical Laboratory (NCL), Pune and maintained by periodical susceptibility on Nutrient Agar and Sabourand Dextrose Agar for bacteria and fungi respectively. These microbial strains are inoculated in peptone water and inoculated at 37^{o}C & 25^{o}C for 6 to 18 hours for bacteria and fungi respectively.

Standardization of Inoculum

Reproducibility of the disc-diffusion test largely depends on the size of the inoculums used. The zone of inhibition decreases with increasing size of the inoculums, because the antimicrobial agent has to react with a greater number of bacteria. Hence the inoculums size should be standardized. Standardization of inoculum is done by comparing with the turbidity of the inoculum. The standard roughly compares with $1x10^8$ organisms/ml, or 2 organisms seen on the smear under oil immersion objective.

Preparation of Standard

Mixed 0.5 ml of 1.175% (w/v) hydrated barium chloride ($BaCl_2$.$2H_2O$), with 99.5 ml of 1% (w/v) or 0.36N sulphuric acid (H_2SO_4). The resultant suspension of barium sulphate precipitate acts as the standard (1 x 108 cells/ml). The standard was distributed in screw capped tubes of the same size as those used in growing the broth culture which contains approximately 4 to 6 ml per tube. Shaken the standard before comparing and replaced the standard every 6 months.

Preparation of Media

For Bacteria

A Muller Hinton Agar plate used for testing by the disc diffusion technique. Prepared the medium by adding the ingredients as in dissolved by gentle heating, pH 7.2 and sterilized by autoclaving at 121^{o}C for 15 to 20 minutes. Cooled the sterile medium at 50^{o}C, poured in large-size petridishes to an uniform depth of 4mm and then allowed the plates to solidify at room temperature.

For Fungi

Sabourand Dextrose Agar plate used for testing by the disc diffusion technique. Prepared the medium by adding the ingredients as in dissolved by gentle heating at pH 6.8 and sterilized by autoclaving at 121^{o}C for 15 to 50 minutes. Cooled the sterilize medium at 50^{o}C. Poured in large-size petridishes to an uniform depth of 4mm and then allowed the plates to solidify at room temperature.

Procedure

- ✓ Labelled the plate with name of the culture, sample, standard and if any specification required.
- ✓ Dipped a sterile cotton swab on a wooden applicator stick into the bacterial suspension.
- ✓ Excess fluid removed by rotating the swab with a firm pressure against the inside of the tube above the fluid level.
- ✓ Rubbed gently over the plate in several directions to obtain uniform distribution of the inoculums.
- ✓ Taken a fine pointed pair of forceps, alcohol flame and cooled. The sterile disc was held with the forceps and placed on the inoculated plate (15 mm from the edge of the plate and 24 mm in between the centre of the discs) about 5 discs can be placed over the 10 cm diameter petridish. Same way followed for placing the standard disc.
- ✓ Taken the micropipette and loaded the sample in the sterile disc carefully (quantity required).
- ✓ Incubated all the plates at 370^{o}C in an incubator with upright position. within 15 minutes after placing the discs.
- ✓ Following incubation, (the diameters of the zones of inhibition of growth (including the 6mm diameter of the discs itself) was measured by using a ruler.

Results are reported as follows

Observation		**Report**
Zone more than 12mm	-	Sensitive
Zone 4 to 12 mm	-	Intermediate
Zone less than 4mm	-	Resistant

PREPARATION OF CULTURE MEDIUM

Muller Hinton Agar (Bacteria)

INGREDIENTS		**QUANTITY REQUIRED**
Beef extract	-	300 g
Peptone	-	17.5 g
Starch	-	1.5 g

Weighed and suspended the ingredients in 1000ml of cold distilled water. Heated to boiling. Adjusted the pH 7.4 and sterilized by autoclaving at 121^{o}c for 10 minutes. Cooled to 50^{o}C and poured in sterile petridish.

Sabourand Dextrose Agar (Fungi)

INGREDIENTS		**QUANTITY REQUIRED**
Dextrose	-	40 gm
Peptone	-	10 gm
Agar	-	20 gm
Distilled water	-	1000 ml

Table - 16

Zone of Inhibition Shown by the Ethanolic extract of Whole Plant of *Sida spinosa* Linn.

S.No	Micro organism	Zone of Inhibition (mm)							
		A	**B**	**C**	**D**	**E**	**F**	**G**	**H**
1	*Staphylococcus aureus* (NCM2079)	12	15	15	15	17	19	-	38
2	*Bacillus subtilis* (NCM 2439)	13	18	20	20	21	23	-	35
3	*E.coli* (NCM 2965)	12	14	16	18	19	21	-	38
4	*Pseudomonas aeruginosa* (NCM 2036)	14	14	15	16	20	22	-	40
5	*Candida albicans* (NCM 3102)	14	15	16	16	18	21	-	32
6	*Aspergillus niger* (NCM 105)	-	-	-	-	-	-	-	34

A- Ethanolic Extract of *Sida spinosa* 50 µg/disc

B- Ethanolic extract of *Sida spinosa* 100 µg / disc

C- Ethanolic Extract of *Sida spinosa* 200 µg/ disc

D- Ethanolic Extract of *Sida spinosa* 300 µg/ disc

E- Ethanolic Extract of *Sida spinosa* 400 µg/ disc

F- Ethanolic Extract of *Sida spinosa* 500 µg/ disc

G- Solvent Control (DMSO)

H- Standard Drug Ciprofloxacin – 5 µg / disc

RESULTS AND DISCUSSION

The present study entitled "Pharmacognostical, Phytochemical and Pharmacological studies on whole plant of *Sida spinosa* Linn. (Malvaceae)

Pharmacognostical Studies

The plant *Sida spinosa* Linn. is an erect, annual or perennial, stellate, 30 cm to 1 m tall under shrub. Leaves with filiform, 2 – 5 mm long stipules, petiole 2 – 20 mm long, 1 – 3 spiny tubercles present on stem at the base of petiole, lanceolate to ovate, oblong or somewhat orbicular, round at base, acute or obtuse at apex, serrate. Flowers axillary, solitary or 2 – 5 in fascicles in terminal branches, pedicel 2 – 5 mm in, fruit upto 0.2 cm long, joined near the middle or top. Calyx 4 – 5 mm long. Fruit depressed globose, pubescent above, mericarps 5, membranous, 2–3 mm long. Seeds 1.5 mm long, glabrous, brown to black.

Microscopy

The leaf, petiole, stem, root of *Sida spinosa* were subjected to microscopical studies. The leaf midrib consists of bowl shaped abaxial part and slightly convex on the adaxial side of the midrib, the palisade parenchyma of the lamina tranverse upto the adaxial part of the midrib, and the ground tissue is inconspicuous in the adaxial state.

Lamina

The lamina is 150 µm thick. The leaf is dorsiventral, mesomorphic, hypostomatic, the adaxial epidermis is single layered and consist of squarish to rectangular; thick walled, epidermal cells.

Petiole

The petiole is cordate shaped in cross sectional outline, with wide cup shaped abaxial part and a flat adaxial side, the epidermis of the petiole is single layered. Petiole is differentiated into outer three or four layers of thick walled, angular, compact, collenchyma cells and inner thin walled, elliptical parenchymatous ground tissue.

Stomatal Morphology

The abaxial epidermis is stomatiferous, the epidermal cells are thick walled and have wavy anticlincal wall, the stomata is anomocytic.

Venation Pattern

The venation pattern of the lamina shows the vein islets and vein termination; in *Sida spinosa* the vein islets are rectangular or square shaped and wide; the vein terminations are fairly distinct, short and branched once or unbranched, the branched termination extend upto the middle portion of the vein islet. The extreme ends of the vein terminals bear small cluster of elongated tracheids.

Stem

The stem is circular in cross section; the stem shows normal secondary growth; the stem has thick hollow, continuous cylinder of secondary xylem and secondary phloem. No periderm is found.

Root

In cross sectional outline the root is circular and shows secondary growth the epidermis is replaced by a narrow zone of periderm; periderm is superficial in origin.

Phytochemical Studies

The whole plant powder of *Sida spinosa* Linn. was extracted with different extracts of increasing polarity ranging from Petroleum ether, Chloroform, Acetone, Ethanol and Water. The extractive values are shown in **Table – 2.**

The physico chemical standard values such as total ash, water soluble ash, acid insoluble ash, sulphated ash, loss on drying, water soluble extractive value, alcohol soluble value, and crude fiber content are reported in **Table - 2.** The alcohol soluble extractive values are higher than water soluble extractive value.

The fluorescence analysis of extracts and powders with different reagents were studied in day light and UV light. The observations are shown in **Table – 3**

All the extracts were subjected to preliminary phytochemical investigation. More number of phytoconstituents like alkaloids, flavonoids, phytosterols were found to be present in Ehanolic extract. Ethanolic extract was subjected to Thin Layer Chromatography (TLC) for alkaloids, glycosides, flavonoids, steroids and essential oil. The number of spots with its R_f values are shown in **Table – 6.**

From the results obtained in Thin Layer Chromatography (TLC), it was observed that more prominent spots was observed for the Ethanolic extract of *Sida spinosa* and were subjected to Column Chromatography.

An yellow crystalline compound was obtained by column chromatography in the fractions of Ethanolic extract (Acetone : Ethylacetate 50 : 50) and was named SS1.

- The isolated compound SS1 was crystalline in nature, yellow in colour, odourless and bitter in taste with melting point (155^0 - 160^0 C), Soluble in Methanol and Ethyl acetate.

- The isolated compound SS1 subjected to FTIR studies for the identification of characteristic functional groups present in the compound. In this studies it showed Phenolic (3828 – 3940 cm^{-1}), Nitro (3501cm^{-1}), Carbonyl (1064cm^{-1}) Aromatic (1405 cm^{-1} O-H Bending, 3000 cm^{-1} O-H Stretching) **(Fig - 22).**

- The isolated compound SS1 subjected to HPTLC studies and the results are shown in **Table – 9, 10, 11, Fig – 23 – 27.**

- To characterize the isolated compound SS1 it was also subjected to GC – MS studies and compared with NIST 2005 library, structures of these constituents are shown in **Fig – 28, 29, 30.**

Molecular weight 139, formula – $C_7H_9NO_2$, (1H – Pyrrole - 2, 5 - dione) it is a nitrogen containing compound. Molecular weight 426, formula– $C_{30}H_{50}O$ (Lupeol) it is a characteristic cyclic nucleus in Steroidal structure. Molecular weight 384, formula – $C_{27}H_{44}O$ (Cholesta - 4, 6 – dien - 3ol) it is poly phenolic compound and commonly known as Steroidal compounds.

Based on the above spectral data, the isolated compound may be constitute a 1H-pyrrole ring (Phenolic), and Lupeol (Steroidal) nucleus, Cholesta (Steroidal) nucleus, FTIR evidenced the presence of phenolic group (3828 – 3940 cm^{-1} O - H stretching).

From the identification test, melting poit studies and spectral analysis it is concluded that the isolated compound SS1 may be a Phenolic/ Steroidal compound.

Pharmacological Activity

Acute toxicity studies revealed the non toxic nature of the ethanolic extract of *Sida spinosa* upto a dose level of 2000 mg/kg body weight in rats. There was no lethality or toxic reaction found at any of the dose selected until the end of the study.

The plant extract of *Sida spinosa* Linn. showed antidiabetic activity by reducing blood glucose level significantly. It was also much effective when compared with the standard drug Glibenclamide. It reduced blood glucose level upto 224.0, 200.3, 182.8 mg dL^{-1} at successive days of 4, 7, 10 at the dose of 400 mg/kg in rats compared with the standard drug which reduced blood glucose level upto 222.0, 175.7, 137.7 mg dL^{1} respectively. The ethanolic extract of plant *Sida spinosa* Linn. was effective in reducing blood glucose level compared to the standard drug (Glibenclamide).

Pancreatic section stained with hematoxylin and eosin (H & E) showed that alloxan caused severe necrotic changes of pancreatic islets, especially in the centre of islets. Nuclear changes, karyolysis, disappearance of nucleus and in some places residue of destroyed cells were visible. Relative reduction of size and number of islets especially around the central vessel and severe reduction of beta cells were clearly seen in diabetic control.

There was also a relative increase of granulated and normal beta cells in the diabetic group which consumed 200 mg/kg body weight of ethanolic extract, when compared with the diabetic group which consumed 400 mg/kg body weight of ethanolic extract of *Sida spinosa* Linn. .

Pancreas of the diabetic group administered with Glibenclamide 10 mg/kg body weight showed close similarity to group which was treated with test extract of *Sida spinosa* Linn.

Antimicrobial Activity

The Antimicrobial activity of Ehanolic extract of *Sida spinosa* were studied against Gram positive, Gram negative bacteria and Fungi (**Table – 16)**

The ethanolic extract of *Sida spinosa* was found to possess a maximum anti bacterial activity as with the standard drug Ciprofloxacin (5 mcg/disc) for bacteria and fungi respectively.

The presence of either flavonoids, phenolic compounds of *Sida spinosa* may be responsible for antimicrobial activity. The results are shown in **Fig. 35 – 41.**

CONCLUSION

In the present study, the plant *Sida spinosa* was selected to explore the scientific information on Pharmacognosy, Phytochemical and Pharmacological aspects.

The parameters which are reported under pharmacognostical and phytochemical studies could be used for botanical identification of the drug in the crude form and preparation of monograph of the plant *Sida spinosa* Linn.

Diabetes mellitus is a heterogeneous metabolic disorder as old as mankind and its incidence is considered to be high (4 - 5%) all over the world. The present investigation has brought the drug *Sida spinosa* Linn. could serve as a better drug for effective treatment against Type II diabetes. Also the plant *Sida spinosa* may represent a new source of antimicrobial with stable, biologically active compounds that can establish a scientific base for the use this in modern medicine.

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